From the Department of Cell and Molecular Biology
Karolinska Institutet, Stockholm, Sweden

REGENERATING THE BRAIN: LESSONS FROM THE RED SPOTTED NEWT

DANIEL BERG

Karolinska Institutet

Stockholm 2012

Published by Karolinska Institutet. Printed by Larserics Digital Print AB
Cover art by Laura Bott

ISBN 978-91-7457-870-6

Betrachtet, forscht, die Einzelheiten sammelt,
Naturgeheimnis werde nachgestammelt.

Observe, investigate, with searching eyes,
And nature will disclose her mysteries.

Goethe

ABSTRACT

Unlike mammals, adult salamanders can regenerate their brain after injury in a process fuelled by neurogenesis. The aim of this thesis is to identify the cells that give rise to new neurons after injury and examine the mechanisms controlling the initiation and termination of brain regeneration in the newt. The reasons why newts can regenerate their brain while mammals cannot are unknown, and it has been argued that the presence of constantly proliferating cells is a prerequisite for regeneration to occur.

In **Paper I** of this thesis we mapped the distribution of proliferating cells in the adult newt brain and identified the areas of the brain where neurons are added under normal conditions. We observed that similarly to mammals, neurons are continuously added to the newt forebrain, while no neurogenesis was detected in the midbrain. After injury to the newt midbrain, radial glia-like cells (RGLs) start to proliferate and progeny of these cells differentiate into neurons. These results show that regeneration is not dependent on constitutive neurogenesis.

In **Paper II** we wanted to test if the neurotransmitter dopamine is involved in controlling regeneration of dopaminergic (DA) neurons. We found that synthetically increasing the concentration of dopamine in the regenerating newt brain by administration of the dopamine precursor L-dopa, inhibits regeneration by blocking the proliferation of the progenitor cells. We also found that antagonizing dopamine signalling in the uninjured brain is sufficient to evoke proliferation of the otherwise quiescent RGLs and we found that progeny of these cells give rise to increased numbers of neurons in the midbrain.

The newt is not the only vertebrate animal able to regenerate its brain; both fish and reptile species can repair injured brain. Interestingly, fish and reptiles are also able to survive hypoxia and are exposed to these conditions in their natural habitat. In **Paper III** of my thesis we wanted to examine if the ability to regenerate brain tissue is linked to an animal's ability to survive varying levels of oxygen. We observed that the red spotted newt is able to survive hypoxia, but this treatment leads to increased cell death in the brain. Reoxygenation of the brain leads to an elevated production of reactive oxygen species, which is concomitant with an increase in proliferation of the ventricular progenitor cells, suggesting that a regenerative process has been initiated. Inhibiting production of reactive oxygen species during reoxygenation results in reduced progenitor cell proliferation.

The major findings of this thesis are summarized as follows: the red spotted newt is able to regenerate areas of the brain that are normally devoid of proliferating cells. In this process, ventricular RGLs act as progenitor cells in the adult newt brain and give rise to new neurons after injury. We have also shown that the neurotransmitter dopamine inhibits the proliferation of DA progenitor cells in a feedback-like manner and thus identified a mechanism for how the brain senses the degree of neuronal loss. These results pinpoint several features of naturally occurring brain regeneration and could aid the development of techniques to evoke brain regeneration in humans. Finally we have found that the newt, similarly to other animals capable of brain regeneration, is able to survive hypoxia. Hypoxia leads to increased cell death in the brain, but molecules that are activated by reoxygenation induce regenerative mechanisms such as increased proliferation of neural progenitor cells. These discoveries suggest that the trait of brain regeneration has evolved together with the capacity to survive variation in oxygen levels.

LIST OF PUBLICATIONS

I. **Daniel A Berg**, Matthew Kirkham, Anna Beljajeva, Dunja Knapp, Bianca Habermann, Jesper Ryge Elly M Tanaka and András Simon (2010)
Efficient regeneration by activation of neurogenesis in homeostatically quiescent regions of the adult vertebrate brain.
Development, 137:4127-4134.

II. **Daniel A Berg**, Matthew Kirkham, Heng Wang and András Simon (2011)
Dopamine controls neurogenesis in the adult salamander midbrain under homeostasis and during regeneration of dopamine neurons.
Cell Stem Cell 2011, 8, 426-433.

III. **Daniel A Berg**, Lasse D Jensen, Yihai Cao and András Simon
Reoxygenation after systemic hypoxia induces tissue damages and activates regenerative mechanisms in the adult newt brain.
Manuscript

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

3AP	3-acetylpyridine
^{3}H	tritiated thymidine
6-OHDA	6-hydroxydopamine
BrdU	5-bromo-2'-deoxyuridine
D1L	D1-like receptor
D2L	D2-like receptor
DA	dopaminergic
Dcx	doublecortin
DG	dentate gyrus
DGC	dentate granule cell
DNA	deoxyribonucleic acid
GABA	γ-aminobutyric acid
GFAP	glial fibrillary acidic protein
GFP	green fluorescent protein
HVC	higher vocal centre
IPC	intermediate progenitor cell
IPS cell	induced pluripotent stem cell
LGE	lateral ganglionic eminence
MCM2	mini-chromosome maintenance protein 2
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	messenger ribonucleic acid
PCNA	proliferating cell nuclear antigen
PEC	pigment epithelial cells
pg	pictogram
RGL	radial glial-like cell
RMS	rostral migratory steam
SGZ	subgranular zone
SNc	substantia nigra pars compacta
SVZ	subventricular zone
TUNEL	terminal deoxynucleotidyl transferase mediated dUTP nick end labelling
UTR	untranslated region

1 REGENERATIVE BIOLOGY

1.1 INTRODUCTION

The capacity of the human body to repair injured tissues and organs decreases with age. At the same time, the likelihood that a person will get degenerative disease increases. Since the population of the industrialised world is rapidly ageing, the number of patients suffering form degenerative diseases will drastically escalate. To cope with this increase in patients, novel methods need to be developed to cure these patients.

An organ that is particularly sensitive to ageing is the brain and many neurodegenerative diseases, such as Parkinson disease and Alzheimer's disease become more prevalent with age. Current treatments of neurodegenerative diseases are usually aimed at alleviating the symptoms caused by degeneration in the brain, not repairing the injury. Possible ways to cure degenerative diseases is to replace the lost cells and injured tissue using stem cells. Stem cells exist in many organs of the human body including the brain and these cells have the capacity to repair organs after natural wear or injury. A great deal of effort in the field of regenerative medicine is now placed on developing methods that utilize stem cells to repair injuries caused by disease or trauma. This can be done either by enhancing the activity of somatic stem cells that are already in the body or by injecting stem cells that have been manipulated in a dish, into the diseased part of the body.

Unlike humans, some non-mammalian vertebrates such as fish and amphibians have proved to be excellent natural regenerators and are able to rebuild various organs without transplantation or injection of stem cells. The most impressive example of these natural regenerators is the newt. This aquatic salamander has been used in regeneration studies for at least 250 years and is known to regenerate limbs, lens, retina and heart tissue after injury. In contrast to mammals, newts shows no age related decline in their regenerative capacity and a lot can be learned of the processes controlling regeneration in this animal. Work done in my PhD group has previously shown that newt is also able to recover from a toxin induced parkinsonian-like condition. After ablation of the midbrain dopaminergic (DA) neurons, which correspond to the neurons that die in patients suffering from Parkinson's disease, it takes approximately 30 days for the neurons to grow back. However, the origin of the cells and factors controlling neurogenesis following injury were not known.

In this thesis I study the cellular source involved the regeneration process of the adult newt brain. I also examine the signals that control the progenitor cells during regeneration. Finally, I examine the reason why newts have developed this amazing ability to regenerate brain tissue. In the background section of this thesis, I will firstly give an introduction to the field of experimental regenerative research, with a section explaining the various mechanisms involved in regeneration in different model organisms. Secondly I will introduce the reader to the process of neurogenesis, both in the injured and un-injured adult vertebrate brain. And finally I will give a brief introduction to the dopaminergic system and diseases caused by changes in this system, and explain how dopamine is known to regulate stem cell behaviour.

1.1.1 Experimental Regenerative biology

The concept of regenerating lost body parts has captured people's imagination through the ages and is well documented in different mythologies of many cultures. Greek mythology discusses Prometheus, the half man half God who was caught by Zeus for having given knowledge of fire and language to the humans. He was condemned to be chained to a rock and having an eagle peck out his liver every night. Every morning the liver had regenerated and Prometheus would have to suffer the same punishment over and over. Remarkably, the human liver is an organ with a relatively good regenerative capacity (Pack et al., 1962). Whether or not the ancient Greeks had performed any experiments or were only incredibly perspicacious, we do not know. The Greek hero Heracles also encountered regeneration when he tried to decapitate the nine-headed Lernaean Hydra. Every time he cut of a head, two new ones replaced it.

Aristotle (384-322BC) the great natural philosopher and scientist, mentions the concept of regeneration in his book "The History of Animals". He states that lizards are able to regenerate their tail and the chick of the swallow is able to repair its eyes. In addition, he discusses the ability of male deer to regenerate their antlers on a yearly basis. He also noted that were unable to regenerate their antlers following castration (Aristotle, 1984). It took over two millennia for scientists to discover that castration of deer affects the androgen levels in the body, which in turn affects the regeneration process (Kierdorf and Kierdorf, 2011; Kierdorf et al., 2004).

Though there were observations of regeneration during ancient times, no actual experimental regeneration studies were reported until the eighteenth century. The pioneer of this work was René Antoine Ferchault de Réaumur (1683-1757). Réaumur was a great observer and experimental scientist, Thomas Henry Huxley (1825-1895) even placed him on par with both Aristotle and Charles Darwin (Huxley and Huxley, 1900), and his work inspired many of his contemporaries (Ratcliff, 2005). Réaumur is most famous for his work in the iron and steel industry, he even got a temperature scale named after him in honour of him inventing the alcohol thermometer. Biologists will nevertheless remember him for his works on the regenerative capacity of crustaceans. In 1712, he published a paper in which he showed that the blackback land crab (*Gecarcinus lateralis*) is able to regenerate amputated limbs.

Réaumur's pivotal regeneration experiment inspired his scientist colleagues to examine if regeneration occurred in other animals (Odelberg, 2004; Ratcliff, 2005). One of these scientists was Abraham Trembley (1710-1784), a Swiss naturalist who

studied regeneration in a fresh-water polyp. He discovered that if this animal is cut into two pieces, both pieces regenerate a new body, the head part regenerates a new foot, and the foot part regenerates a new head. Because of these animals striking resemblance to the monster Hercules fought with, Carl Linnaeus (1707-1778) decided to name them Hydra (Oxford English Dictionary). Charles Bonnet (1720-1793), who happened to be Trembley's cousin, performed similar experiments on worms. He observed that worms also had the power to regenerate and he showed that they could even remake complete animals from the small parts that were cut from the animals (Birnbaum and Sanchez Alvarado, 2008; Tsonis and Fox, 2009). Apart from studying regeneration, Bonnet also contributed to the field of biology by identifying another interesting biological phenomena called Parthenogenesis. Parthenogenesis is a form of asexual reproduction that Bonnet observed while studying the plant lice (aphids) (Davis, 2012; Dinsmore, 1991; Suomalainen, 1950). This phenomenon is now known to exist in other vertebrates species including some amphibians and reptiles (Lampert, 2008). Interestingly, the capacity to reproduce asexually is often thought to be linked with the capacity to regenerate damaged tissue (Sanchez Alvarado, 2000).

The first person to do systematic experiments on salamanders was Lazzaro Spallanzani (1729-1799). He reported in 1768 that salamanders are able to regenerate their limbs and tails after amputation. He also observed that upon amputation of a limb, a thin round or elliptic surface was formed at the site of injury. Today we know that this surface, also called the blastema, consists of a cell mass that gives rise to the regenerate (Tsonis and Fox, 2009). For their scientific achievements Réaumur, Bonnet and Spallanzani were given several awards and were all elected foreign members of the Royal Swedish Academy of Sciences (Dahlgren et al., 1915).

The discovery that adult animals can rebuild organs *de novo* (from the beginning) had great implications for the way people thought about nature and also affected several natural philosophers of the eighteenth century. One of the central biological questions at this time was whether an organism develops through epigenesis, or preformation. These terms are eloquently explained in a footnote to the English translation of Aristotle's, "The Generation of Animals": *"Does the embryo contain all its parts in little from the beginning, unfolding like a Japanese paper flower in water (preformation), or is there a true formation of new structures as it develops (epigenesis)?"* (Aristotle, 1943).

The discovery of regeneration would convince many that epigenesis was the correct alternative since it appeared improbable that the newt for example, had small auxiliary limbs in the body that could start to grow in case of injury. Though it should be noted that not everyone was convinced and even Réamur and Bonnet remained preformationists (Tsonis and Fox, 2009). In 1856 Charles Darwin (1809-1882) published his pivotal "On the Origin of Species". Though the concept of regeneration was not mentioned in the book, the discovery of evolution and natural selection opened up the possibility to ask a whole new set of questions when discussing regeneration. One of these questions that was hotly debated in the late nineteenth century was whether regeneration is an adaptive feature in animal evolution or if it is a capacity intrinsic to metazoan life, but lost during in animals when they adapt to various environments and behaviours. The two main protagonists is this debate were August Weismann (1834-1914) and Thomas Hunt Morgan (1866-1945), two giants of developmental biology in the late nineteenth century and early twentieth century (Goss, 1992).

Weismann is most famous for his "germ plasm theory" in which he proposes that germ cells contain the heritable information of an organism, while somatic cells perform regular bodily functions. But Weismann also had a great interest in regeneration. He stated that the power to regenerate various organs would have to be introduced by nature since it is a trait that is highly beneficial to the organism in question. Thomas Hunt Morgan on the other hand argued that since regeneration is a very complex process, it would be a highly improbable that it would evolve over-time. Rather, he argued, regeneration is a property inherent to metazoan life, but the capacity to regenerate is lost in some animals when they adapt to their special niche (Goss, 1992). Morgan also argued that if regeneration was an adaptive trait, developed in animals to repair organs that are liable to injury, animals should not be able to regenerate organs that are not likely to be injured. He tested this hypothesis in the common hermit crab *(Eupagurus longicarpus)* and he observed that this animal could regenerate appendages on the anterior part of the body, that are usually protected by the shell and thus not liable to injury (Morgan, 1901b). It should be noted that these experiments have later been repeated with varying results (Goss, 1992).

The question whether regeneration is an adaptive trait or inherent to multicellular life is still debated and will be further discussed in this thesis (Agata and Inoue, 2012; Bely and Nyberg, 2010; Brockes et al., 2001; Garza-Garcia et al., 2010).

1.1.2 What is an adult?

Before a discussion on adult regeneration and adult neurogenesis can commence, it is important to define what an adult actually is. When comparing species from different animal classes, it can be complicated to give definitions. An animal is sometimes considered to be adult when it reaches sexual maturity, but complications can arise when dealing with animals that have unconventional life cycles. For example, some animals die naturally without reaching sexual maturity. This is the case with animals that reproduce asexually, such as animals that replicate solely by parthenogenesis (Schwander et al., 2011). Further examples of animals that do not become sexually mature can be found amongst the insects, such as worker ants that are members of an ant colony and remain sterile throughout their life (Jemielity et al., 2005). So by this definition these animals never become adults. Complications also arise when studying amphibians. Amphibians usually pass through metamorphosis before becoming sexually mature, but there are exceptions. The Axolotl (Ambystoma mexicanum) does not usually metamorphose, but becomes sexually mature while retaining features that in other amphibians are restricted to juvenile animals, such as keeping their external gills and their caudal tail fins (Rosenkilde and Ussing, 1996). This concept is called paedomorphosis and occurs in some species of amphibians (Laudet, 2011).

In this thesis I will mostly discuss vertebrates and I will define an adult animal as an animal that has reached sexual maturity.

1.2 METAZOAN REGENERATIVE BIOLOGY

1.2.1 Morphallaxis vs. Epimorphosis

Thomas Hunt Morgan divided regeneration into two major classes depending on specific criteria; morphallaxis and epimorphosis (Morgan, 1901a; Sanchez Alvarado, 2000). Morphallaxis is defined as a regenerative process that is not fuelled by proliferating cells, but rather the re-organisation of pre-existing cells while epimorphosis is when progeny of proliferating cells give rise to the regenerate.

An animal, commonly used as an example of morphallacitc regeneration, is the Hydra, in which the injury-induced morphogenesis mostly arises from myoepithelial cells (Fujisawa, 2003; Holstein et al., 1991). Though the Hydra can regenerate when mitosis is inhibited (Park et al., 1970), recent discoveries, however, indicate that there is a proliferation zone in the regenerating hydra, which plays a important part in the regeneration process (Chera et al., 2009), thus excluding the hydra from the group of morphallactic regenerators, in a strict sense of the word. Another animal that is considered to regenerate partly through morphallaxis is the planarian flatworm. This worm of class turbellaria, was shown by Morgan, to be able to regenerate a whole animal from 1/279th piece of the body (Morgan, 1898). Therefore, Morgan considered planarians to regenerate through morphallaxis, but recent research has found that proliferation of neoblasts, pluripotent stem cells in the Planaria, is required for regeneration (Reddien and Sanchez Alvarado, 2004; Wagner et al., 2011). So there are examples where morphallaxis plays a part of the regeneration process, in the sense of remodelling of tissue. But in most regenerative animals studied, proliferation of cells is a necessity for complete regeneration to proceed. It is also interesting to note that animals who have a constant number of cells as adults such as nematodes (roundworms) and Rotifera (wheel animals) appear to lack regenerative capacity, suggesting that constant cell turn-over is a requirement for regeneration (Hughes, 1989; Sanchez Alvarado, 2000).

Considering that no animals appear to be able to regenerate tissue completely without a proliferative response, morphallaxis in the strict sense appears to be a somewhat out-dated term (Agata et al., 2007). Although tissue reorganisation does occur during regeneration, all cases of functional organ regeneration should in my mind be considered to be epimorphic.

1.2.2 Epimorphosis and the origin of the cells that fuel regeneration

Epimorphosis, by Morgan's definition, is regeneration where proliferation of cells precedes and interacts with the process of new tissue formation. Cases of epimorphic regeneration are found throughout the metazoan kingdom and interesting examples can be found in various types of animals, such as insects (Anderson and French, 1985), worms (Wagner et al., 2011) and starfish (Thorndyke et al., 2001). Though these model organisms are of great importance for the field, this thesis will mostly focus on epimorphic regeneration in vertebrates.

The proliferating cells that give rise to the new tissue in different cases of epimorphic regeneration originates from different sources. The cells can originate from trans-differentiation of mature cells, from cells that have been dedifferentiated or from somatic stem cells that reside in the body. In this section I will explain these concepts and discuss differences and similarities between these different mechanisms.

1.2.2.1 Transdifferentiation

When the lens of the urodele amphibian is injured or removed, pigmented epithelial cells (PEC) originating from the dorsal iris start to loose colour and alter their shape. PECs start to proliferate and subsequently change phenotype, eventually giving rise to a new lens. This process is called trans-differentiation (Figure 1A) (Jopling et al., 2011; Zhao et al., 1997). In mammals, there are examples of transdifferentiation occurring during development. For example in the development of the oesophagus, where smooth muscle cells in the developing animal has been found to switch to skeletal muscle phenotype during the first period after birth (Patapoutian et al., 1995). Transdifferentiation as a process can take place in the absence of proliferation, which is the case when pancreatic exocrine cells are experimentally transdifferentiated into beta cells, by ectopic expression of three transcription factors (Zhou et al., 2008).

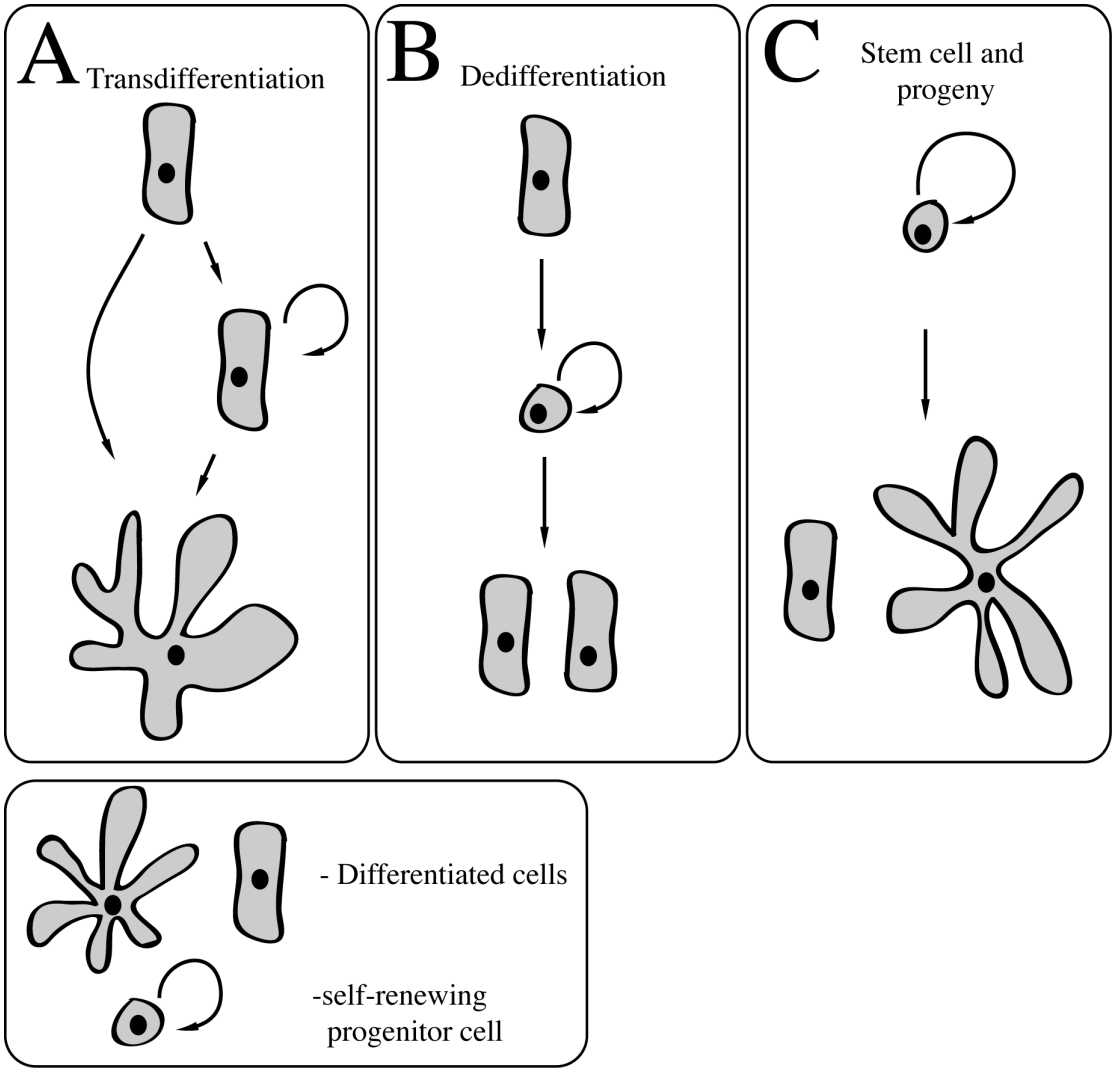

Figure 1: Different origins of cells that contribute to the regenerating tissue.
A) Cells that undergo transdifferentiation change from one type of differentiated cell into another, this can take place both with or without proliferation taking place.
B) Dedifferentiatiated cells loose their differentiated character and adopts characteristics of a proliferating progenitor cells that can differentiate back into specialized cells. **C)** Stem cells have the capacity to self-renew and give rise to one or more types of differentiated cells.

1.2.2.2 Dedifferentiation

Dedifferentiation is the process by which a terminally differentiated cell looses its differentiated characteristics and becomes a proliferating progenitor cell (Figure 1B) (Tanaka and Reddien, 2011). A classic example of dedifferentiation has been observed in the regenerating newt limb. When muscle fibres in the newt limb become injured, the multinucleated muscle fibres (syncytium) start to fragment into mononucleated cells (Charge and Rudnicki, 2004; Echeverri et al., 2001). These cells have the capacity to re-enter the cell cycle and give rise to parts of the blastema, a mass of proliferating cells

that eventually grows and becomes a new limb (Kumar et al., 2000; Tanaka and Reddien, 2011). Blastema formation is a central process in limb regeneration, but it should be noted that the definition of blastema varies between model organism and scientist, making it a rather confusing concept. Sometimes a blastema is defined as a self-organizing clump of proliferating cells that can be transplanted onto other body parts and still give rise to the same regenerate. Other times, a blastema is loosely defined as a group of multipotent cells, making them a common feature in regenerative biology (Brockes and Kumar, 2008; Echeverri and Tanaka, 2005; Poss, 2010; Stocum, 1968).

Other examples of dedifferentiation can be observed in the regenerating heart tissue. Unlike mammals, teleost fish (for example zebrafish (*Danio rerio*)) and amphibians have the capacity to regenerate heart tissue after partial amputation of the ventricle (Poss et al., 2002; Witman et al., 2011). In zebrafish, it has been shown, through genetic trace mapping, that upon injury differentiated cardiomyocytes detach from one another, dissemble their sarcomeric structure and start to enter the cell cycle. Progeny of these cells then differentiate into new functional cardiomyocytes, and functional regeneration of the heart can proceed (Jopling et al., 2010).

In mammals, dedifferentiation is a rare event, but there are some examples of dedifferentiation in a regenerative context. In the peripheral nervous system for example, the nerve projections (or axons) are protected by a sheath of myelin producing Schwann cells. Mature Schwann are normally terminally differentiated and do not proliferate. But if there is an injury to the axon that they are in contact with, the Schwann cells loose this contact and they re-enter the cell cycle. But before this process occurs the mature Schwann cells start to express genes that are associated with immature Schwann cells. Progeny of those cells then develop into mature, myelin-producing Schwann cells (Chen et al., 2007; Muller and Stoll, 1998; Woodhoo et al., 2009).

It is also possible that astrocytes in the central nervous system dedifferentiate after injury to the brain such as ischemia. Upon ischemia astrocytes become reactive and start expressing the neural stem cell marker nestin, though whether these cells give rise to new neurons is yet to be determined (Duggal et al., 1997; Lendahl et al., 1990).

Whilst natural dedifferentiation is a relatively rare event in adult mammals, recent developments have researchers to reprogram mature mammalian cells *in vitro* in a process that could be classified as dedifferentiation. Work lead by Dr. Shinya Yamanaka has shown that overexpressing four transcription factors in cultured

postnatal fibroblasts is sufficient to dedifferentiate them into pluripotent embryonic cell like cells (these cells are known as induced pluripotent stem cells (IPS cells)) (Takahashi and Yamanaka, 2006).

1.2.2.3 Regeneration fuelled by somatic stem cells

A stem cell is defined as a cell that can self-renew, through mitosis, and has the ability to give rise to specialized cell types (Figure 1C) (Gage, 2000; Hsu and Fuchs, 2012; Morrison and Spradling, 2008; Potten and Loeffler, 1990). In mammals the zygote can be considered the ultimate stem cell in that it is a totipotent stem cell. A totipotent is a cell that has the ability to give rise to all cell types in an organism, including the extra-embryonic tissues. The cells that make up the inner cell mass of the developing embryo are considered to be pluripotent. This means that they can give rise to all cells in the embryo (Jopling et al., 2011). Somatic stem cells that reside in the adult body are not pluripotent, but rather multipotent or unipotent, meaning that they can make give rise to not all, by some cell lineages or only one type of cell, respectively (Hsu and Fuchs, 2012).

1.2.2.4 Regeneration by stem cells in non-mammalian metazoans

A remarkable animal that makes use of totipotent stem cells in its regeneration process can be found in the phylum Platyhelminthes or flat worms. The planarian is a bilateral worm that has the capacity to regenerate whole bodies from small body-parts (Morgan, 1898). The only cell type that proliferates in the planarians, whether during regeneration or in the intact animal, is the neoblast. Neoblasts, which are found throughout the body of adult planarians are pluripotent stem cells, since they can generate all cells in the animal, including new neoblasts (Tanaka and Reddien, 2011).

If an animal is irradiated, this kills the neoblasts and the animal cannot regenerate and eventually dies. Recent elegant work has shown that injection of a single, labelled neoblast, into irradiated animals is sufficient to restore the animal's regenerative capacity and rescue the animal (Wagner et al., 2011). Stem cells are also involved in the regeneration in newts. In the limb for example, satellite cells, the muscle stem cells involved in growth and regeneration of muscle tissue, are found in close contact with muscle. Upon amputation of a newt limb, these satellite cells re-enter the cell cycle and give rise to new muscle. Newt satellite cells that have been cultured *in vitro* and, when injected into a regenerating limb, are even able to give rise non-muscular tissues such as cartilage and epidermis (Morrison et al., 2006). Interestingly, lineage crossing of

satellite cell progeny has not been observed in regenerating axolotl limb, suggesting that there might be species-specific differences in the mechanisms of limb regeneration and stem cell properties (Kragl et al., 2009).

1.2.2.5 Stem cells in mammals

Mammals do not possess the same capacity to regenerate damaged tissue as planarians or the urodele amphibians, but they do have stem cells. These cells are in charge of tissue repair and maintenance during the animal's lifetime. In the adult mammal, somatic stem cells are found in various tissues, such as brain, skin, intestine, the haematopoietic system and muscle. Some stem cells are only responsible for repair tissue after injury, while others are part of daily upkeep of the tissue. Satellite cells, for example, are adult skeletal muscle stem cells. These cells are usually quiescent, but upon injury they re-enter the cell cycle and produce myoblasts that can fuse with the myofibers and regenerate the muscle (Collins et al., 2005). In tissues where cells are rapidly renewed, such as in the intestine and epidermis, the stem cells divide constantly. The turnover time of the epidermis in the intestine is thought to be 5 days, and it is estimated that stem cells in this organ divide once every day (Barker et al., 2007).

1.2.2.6 How stem cells keep their stemness

The role of somatic stem cells is to maintain and repair tissues for the entire life of the organisms, and for the stem cells to survive for this long period of time, they need to be kept under appropriate conditions. The milieu that stem cells reside in is usually denoted as the stem cell niche. Each niche creates an environment that is beneficial for the respective stem cells type and this environment varies between different populations. The surrounding cells in the niche can communicate with the stem cells through secreted factors and cell surface receptors which influence various aspects of cell behaviour, such as proliferation, fate choice and differentiation.

Stem cells also have intrinsic mechanisms to reduce damage, increase survival and prevent the onset of cellular senescence. While somatic cells are known to divide a finite number of times before becoming senescent, a limit called the "Hayflick limit" (Hayflick and Moorhead, 1961), some stem cells can proliferate throughout the lifespan of the animal, thus defying the "Hayflick limit" (Rando and Chang, 2012). It has been calculated that the intestinal stem cells of mice divide 700–1000 times in the lifetime of the animal (Barker et al., 2007). These cells have been shown to have relatively high level of telomerase activity, an enzyme that restricts the shortening of telomeres

invariably associated with every round of cell division (Schepers et al., 2011). When telomeres length reaches a critical limit, DNA damage pathways become activated in the cells and they become senescent (Sahin and Depinho, 2010). Interestingly telomerase activity appears to be low in some stem cell populations, suggesting that other methods of reducing telomere shortening might be active in these cells (Hiyama and Hiyama, 2007).

In 1975 John Cairns hypothesized that stem cells in order to protect themselves from mutations, might retain the same chromosome strand in each cell division (Cairns, 1975). This theory is usually called the "immortal strand theory" and is difficult to prove or disprove. Thanks to recent technical advancements, its been shown that chromosomes are non-randomly segregated in the intestine progenitor cells and in muscle satellite cells (Falconer et al., 2010; Quyn et al., 2010; Rocheteau et al., 2012). Asymmetric segregation of chromosomes has also been observed in neural stem cells cultured *in vitro,* but there is no evidence supporting the same concept *in vivo* (Karpowicz et al., 2005).

What about cells in the vertebrate species that are capable of regenerating their organs, such as fish and salamanders? How are these hindered from becoming senescencent? Interestingly, myogenic cells taken from newts do not to become senescent when cultured *in vitro*, unlike their rodent counterparts (Ferretti and Brockes, 1988).

But immortalisation of rodent fibroblasts is possible by expressing newt cDNA in these cells. Expression of the 3′-UTR sequence of α-glucosidase related mRNA, isolated from the newt blastema resulted in cells that overcome the Hayflick limit (Powell et al., 1998). Apart from these studies, the mechanisms of senescence avoidance have not been extensively studied in urodele amphibians, though there are some studies in other regenerating animal models. Up-regulation of telomerase activity has been observed in regenerating zebrafish fins and it would be very interesting to examine telomerase activity and chromosome segregation in newt cells (Anchelin et al., 2011).

1.3 THE NEWT AS EXPERIMENTAL MODEL

1.3.1 Ecology and Habitat

Newts are aquatic vertebrates that belong to the subfamily of amphibians called Urodelea or salamanders. The urodeles are classified as amphibians that retain their tails after metamorphosis, unlike anuran amphibians (frogs) that loose their tail during metamorphosis. Newts have a complex life cycle, with three distinct life stages: the larval or tadpole stage, juvenile or eft stage, and the adult stage.

During the larval stage the animals remain entirely aquatic and do not leave the water. Newt larvae have external gills and eventually develop small limbs. During larval metamorphosis, the newts lose their gills and start to develop lungs, a prerequisite for most terrestrial vertebrate life (Shi and Boucaut, 1995). When larval metamorphosis is complete the animals leave the water and start their juvenile life on land. The colour of the animals changes from a brownish-green to a reddish colour and they are now called “Eft” (the word “Eft” is thought to be the linguistic progenitor of the word “newt”, but is nowadays only used when talking about the juvenile animal (Brockes and Kumar, 2005)). The juvenile stage lasts for 1 to 3 years and during this stage the animal may migrate far distances until they find a suitable aquatic habitat (Hurlbert, 1969). When the animals have re-started life in a pond, they then undergo a second metamorphosis after which they are sexually mature and have now changed their colour to a greenish grey with red dots (Brockes and Kumar, 2005). In the adult stage, newts live primarily in water, which is a distinguishing feature of newts compared to other salamanders, which stay on land as adults. During winter, the newts stay in water, and remain active even if the pond they live in becomes ice-covered (Berner and Puckett, 2010).

There are three species of newts that are commonly used in experimental biology; the red- spotted newt (*Notophthalmus viridescens)* from North America, Iberian ribbed newt (*Pleurodeles watl*) found on the Iberian peninsula and the north part of Morocco, and Japanese Fire belly newt (*Cynops pyrrhoghaster*) which is found on mainland Japan (Hillman et al., 2009). The Axolotl (*Ambystoma mexicanum*) is another salamander species often used in experimental regenerative research. It is sometimes mistaken for a newt since it lives in water as an adult. But it is a rather a paedomorphic salamander. In nature, this species does not naturally proceed through metamorphosis, but becomes sexually mature as an aquatic larva (Laudet, 2011).

1.3.2 Regeneration and the Newt

There is a long tradition of using salamanders in experimental biology that began with previously mentioned Lazzaro Spallanzani. He showed that newts regenerate limbs, jaws and tails after they had been surgically removed or injured (Sanchez Alvarado, 2000; Tsonis and Fox, 2009). Since the early work of Spallanzani and others, newts have been of great interest to experimental biologists, but they have not only been used by scientists who study regeneration. These animals possess other qualities making them good models to study for example development and evolution that will be explained below.

Newts are considered to be the champions of regenerators amongst vertebrates and, apart from the aforementioned limbs (Brockes, 1997) and tail (Iten and Bryant, 1976), this animals is able to regenerate spinal cord (Davis et al., 1990), brain (Minelli et al., 1987; Parish et al., 2007), lens (Eguchi, 1988; Tsonis et al., 2004), parts of the heart (Witman et al., 2011), intestine (O'Steen, 1958), upper and lower jaw (Goss and Stagg, 1958) and male but not female gonads (Scadding, 1977).

Interestingly there are some organs the newt cannot regenerate for example the kidney. While kidney regeneration does take place in some vertebrate species, for example gold fish (*Carassius auratus*) have been shown to regenerate renal tubes after toxic insult or partial nephrectomy, the newt is not able to regenerate this organ (Elger et al., 2003; Reimschuessel et al., 1990). When 15 -30% of the kidney is removed from the newt, there is a proliferation response, but no nephrogenesis (Scadding and Liversage, 1974).

There does not appear to be an age dependent decline in the regenerative capacity of newts (Kara, 1994). The process of lens regeneration for example, has been repeated 18 times in the same animal without any observable decline in regenerative capacity. The age of the animals in this experiment was estimated to be at least 30 years, indicating that age does not affect the regenerative capability in this species (Eguchi et al., 2011).

1.3.3 Salamanders in other fields of research

Apart from being exceptional regenerators, there are also additional traits of the salamander that makes it an excellent model for the study of other biological phenomena. The salamander zygote is very large and the development of the embryo takes place in water outside the parental body. These abilities make the newt embryo useful animal model to study embryological development. Hans Spemann (1869-1941) and his student Hilde Mangold (1898-1924) used the embryo of the salamander when they discovered the early amphibian embryos "organiser centres", which were found to control neural induction in the ectoderm (Figure 2A). The discovery of these centres, which are now called Spemann's organizer, were a pivotal discovery in the field of developmental biology, and earned Spemann a Nobel prize (De Robertis, 2006).

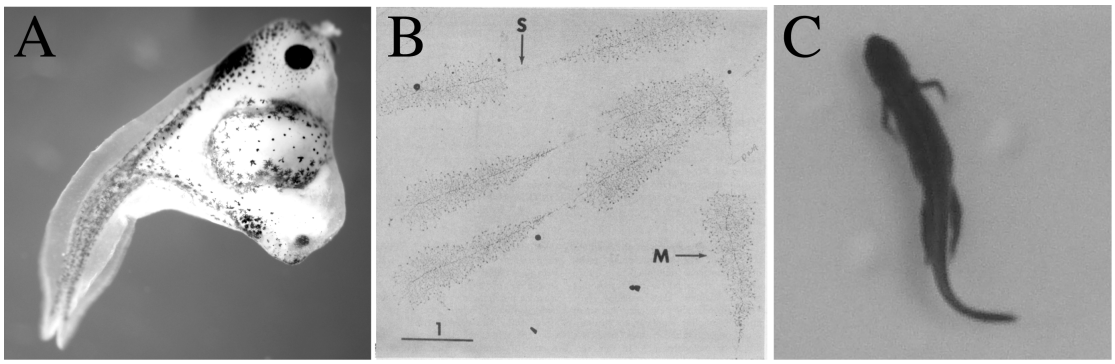

Figure 2: Examples of how salamanders have been used in biological research.
A) Two-headed amphibian larvae after repetition of a classical experiment, firstly performed by Spemann and Mangold in 1924. Photograph by Raju Tomer. **B)** Electron micrograph showing ribosomal DNA transcription on salamander lampbrush chromosomes taken from growing oocytes. From (Miller and Beatty, 1969). Reprinted with permission from AAAS. **C)** A swimming Red spotted newt. Note the S-shaped curvation of the body and how limbs are folded backward against the body. Photograph by Shahul Hammeed.

The large size of the haploid salamander genome makes it a useful model to study cell division and chromosomes. The red spotted newt, for example, have 11 pairs of chromosomes with a total weight of ~39 pg compared to ~3.5 pg which is the genomic weight of human diploid cells (Sexsmith and Toronto, 1968). Walther Flemming (1843-1905) took advantage of the large size of the salamander cells when he discovered the Lampbrush chromosome, a type of chromosome organisation found in oocytes of amphibians and birds (Flemming, 1882; Morgan, 2002). These chromosomes have been useful when studying amongst other things DNA transcription, and Oscar Miller (1925-2012) was able to take remarkable electron micrographs of DNA transcription on the Lampbrush chromosomes using electron microscopy (Figure 2B) (Miller and Beatty, 1969).

In 1985, ten Iberian ribbed newts became some of the first amphibians to visit space. They shared a spacecraft with two rhesus macaques (*Macaca mulatta*) ten male albino rats (*Rattus norvegicus*) and some drosophila flies (*Drosophila melanogaster*) (Desplanches et al., 1990; Gazenko and Ilyin, 1986). Experiments on the newts were aimed to examine if regeneration was affected during and after space travel (Marthy, 2003).

Salamanders are considered to be the animals most closely resembling the first land-living vertebrates and they retain some of the central properties of this putative first terrestrial vertebrate (Frolich and Biewener, 1992; Gao and Shubin, 2001). For this reason salamanders have been used when studying central pattern generators that control locomotion and the evolution of terrestrial locomotion. These animals live both on land and in water and they can move with ease in both settings. When salamanders move in these environments, they use two distinct and natural modes of movement, swimming and walking. These two ways of locomotion activate different movement patterns of the animal's vertebrae. Salamanders swim in a similar way to snakes or primitive fish like the lamprey. While they swim they hold their limbs close to their body and the movement of the trunk is characterized by the use of axial muscles that produce a wave that travels posteriorly through the body and increases in amplitude towards the tail (Figure 2C) (Cabelguen et al., 2010). In **Paper II** of this thesis we take advantage of the newts swimming capacity in a behavioural test to examine injury degree. In this test we measure movement by quantifying the number of times the tip of a newt's tail crosses a line in a grid while swimming.

When on land, salamanders walk using mainly their limb muscles, to create a slow stepping gait by simultaneously moving the diagonally opposed limbs. They can

switch rapidly between swimming and walking. Electrophysiological studies have been performed on salamanders to decipher how the neuronal networks control different modes of locomotion (Cabelguen et al., 2010; Chevallier et al., 2008; Grillner and Jessell, 2009). Recently, a mechanical robot has been generated to model the neural origin of the switch between walking and swimming in the salamanders (Ijspeert et al., 2007).

Another remarkable feature of salamander is that they possess a great lifespan in relation to their size. The olm *(Proteus anguinus)* for example, is thought to have an average life span of at least 68 years and become sexually mature 16 years after birth (Voituron et al., 2011). There is usually thought to be a positive correlation between longevity and body size, but this 25 cm long, blind cave living salamander is obviously an exception to this rule (Finch and Austad, 2011; Speakman, 2005; Voituron et al., 2011).

The salamander that has been used in experiments presented in this thesis is the red spotted newt (*Notophthalmus viridescens)* native to eastern North America. Though it does not have the extreme lifespan of the olm, in captivity red spotted newt have been kept alive for up to 25 years but average lifespan in the wild is estimated to be 12-15 years (Hillman et al., 2009).

2 NEUROGENESIS IN THE ADULT BRAIN

2.1 HISTORIC OVERVIEW

"Once the development was ended, the founts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centres the nerve paths are something fixed, ended and immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree."

-Santiago Ramón y Cajal, 1928

As this quote illustrates, the great neuroscientist Cajal (1852-1934) could not envisage that neurogenesis, the creation of functional neurons took place in the adult brain and he was not alone with this opinion. This was in fact held as a dogma for most parts of the 20th century, and neurogenesis was thought to take place only during embryonic and perinatal life stages of mammalian development but not in adults (Colucci-D'Amato et al., 2006; Gross, 2009; Rakic, 1985). The experimental basis for this theory was that the mammalian adult brain lacked neurons with mitotic figures and the apparent inability of the mammalian brain to regenerate itself after trauma (Altman and Das, 1965).

In the 1950s a new technique was developed, in which the nucleotide Thymidine was synthesised using the radioactive form of hydrogen (tritium or ^{3}H) instead of regular hydrogen. Plants (in this case the English broad bean) were then grown in a medium supplemented with tritiated thymidine, which incorporates into cells that are in the S-phase of the cell cycle, the stage when new DNA is synthesised. Incorporated tritium was then visualized using autoradiography (Taylor et al., 1957). This method gave scientists a tool with which to quantify proliferation and trace the progeny of the proliferating cells. This technique was later transferred to animals (Hughes et al., 1958), and in the 1960s Joseph Altman and colleagues used it to study neurogenesis in the adult rodent brain. Progeny of proliferating cells was observed in the dentate gyrus of the hippocampus and in the subependymal layer of the lateral walls of the lateral ventricles. Altman also observed progeny in the olfactory bulb and in the rostral migratory stream, a rostral extension of the ventricles leading to the olfactory bulb.

Importantly, radiolabelled neurons were also detected, indicating that neurogenesis had in fact taken place in these areas (Altman, 1962, 1969; Altman and Das, 1965).

Despite these initial, yet thorough investigations, the concept of adult neurogenesis did not receive a lot of attention at the time. Work done in non-human primates indicated that new neurons were not added to the brain in the adult primate brain, and it took further technical developments to push the field forward (Rakic, 1985). The development of 5-bromo-2'-deoxyuridine (BrdU), another synthetic thymidine analogue, was very important for the future studies of adult neurogenesis (Gratzner, 1982). Like tritiated thymidine, BrdU can be administered to animals or cells but importantly, it can also be detected by immunohistochemistry. The phenotype of the $BrdU^+$ cells can thus be analysed by co-labelling with other specific antibodies. It should be noted that BrdU has been reported to incorporate into the DNA of cells that are going through cell death, undergoing DNA damage repair or have aborted the cell cycle (Taupin, 2007). It has also been reported that high concentrations of BrdU induces cell senescence in progenitor stem cells (Ross et al., 2008). To circumvent these difficulties it is important to perform co-labelling of BrdU and other proliferation markers such as proliferating cell nuclear antigen (PCNA) and Mini Chromosome Maintenance protein 2 (MCM2) to assess whether the $BrdU^+$ cells are going through the cell cycle.

Another now commonly used technique to trace progeny of dividing cells in the nervous system is *in vivo* administration of replication-incompetent retrovirus (Price et al., 1987). Retroviruses only infect dividing cells, and if the retroviral vector expresses a fluorescent tracer such as green fluorescent protein (GFP), it makes for a very convenient method to visualise the progeny of the progenitor cells in various neurogenic niches (Carleton et al., 2003; Lewis and Emerman, 1994; van Praag et al., 2002).

A caveat with studying stem cell populations using thymidine analogues and retrovirus is that they predominantly label highly proliferating cells. Many stem cells are thought to be mostly quiescent and it is likely that BrdU and retroviruses mostly label transiently proliferating cells (Hsu and Fuchs, 2012). To overcome this problem, lineage-tracing techniques have been developed in which one uses genetic markers to permanently label quiescent stem cells and their progeny (Barker et al., 2007; Bonaguidi et al., 2011).

The concerted use of BrdU, retrovirus and genetic fate mapping techniques, together with technical advancements made in confocal, electron and multi-photon

microscopy and electrophysiological recordings of adult born neurons has pushed the field of adult neurogenesis forward tremendously. We now have a great knowledge of the progenitor cells that reside in the mammalian and non-mammalian brain and the field of adult neurogenesis is rapidly progressing.

In most studied mammalian species, adult neurogenesis occurs in two distinct areas of the adult brains; the subgranular zone (SGZ) of the dentate gyrus in the hippocampus and the subventricular zone (SVZ) of the lateral wall of the lateral ventricles. However, it should be noted that variations exist between species, and there is some controversy concerning the presence of neurogenesis in other areas.

2.1.1 Adult neurogenesis in non-mammalian vertebrates

While adult neurogenesis has been a controversial topic when talking about mammals, it has been less so when dealing with other vertebrates. New neurons are constantly added to the brain of fish, amphibians and reptiles and birds and these species are good models for comparative studies of the mechanism and functionality of adult neurogenesis.

Brain morphology varies greatly between different vertebrate species. The telencephalon in teleost fish for example, develops by outward bending or eversion, while the telencephalon of amniotes and amphibians develop by a process of evagination, resulting in striking structural differences of the ventricular system (Figure 3) (Northcutt, 1981). Despite these differences, the major brain areas are found in most animals, and proliferating, ventricular cells have been observed throughout the rostrocaudal axis of brain of non-mammalian vertebrates.

Studies using tritiated thymidine or BrdU have shown that proliferating cells give rise to cells that express makers for mature neurons or possess the morphology of mature neurons in fish (Adolf et al., 2006; Grandel et al., 2006; Zupanc et al., 2005), amphibians (Polenov and Chetverukhin, 1993; Richter and Kranz, 1981) reptiles (Lopez-Garcia et al., 1988; Perez-Canellas et al., 1997) and birds (Alvarez-Buylla et al., 1990; Goldman and Nottebohm, 1983).

The origin of the new neurons in non-mammalian brains has been studied and is thought to be radial glia-like cells (RGLs), though this has not been shown conclusively. RGLs are sometimes referred to as ependymoglia cells when talking about the amphibian brain. In contrast to the mammals, the brain of fish, amphibians, reptiles and birds retain a large population of RGLs that are found lining the ventricles

throughout the adult brain. RGLs in fish and amphibians are large, bipolar cells that are in contact with both the ventricular and the pial surface of the brain. Their cell bodies are located proximal to the ventricle and they have radial processes spanning through the parenchyma to end in a bushy process that is in contact with the pial surface (Bystron et al., 2008; Rakic, 1971). In mammals, radial glial cells with pial end-feed are known to be neural progenitors in the developing brain, but are absent in the adult brain (Bonilla et al., 2008; Noctor et al., 2001). Mammalian radial glia cells express intermediate filament makers such as nestin, vimentin and GFAP (though GFAP is not expressed in the developing rodent brain (Mori et al., 2005)), and give rise to the subventricular astrocytes (Merkle et al., 2004).

In the teleost fish, new neurons are added throughout the rostral caudal axis of the brain. Neurons are added to the olfactory bulb, large parts of the telencephalon, hypothalamus and cerebellum (Chapouton et al., 2007; Grandel et al., 2006; Zupanc et al., 2005). Though adult neurogenesis has been reported in amphibians, it has not been mapped as extensively as in fish. Neurogenesis has been reported in the hypothalamus and preoptic nucleus of frogs (Chetverukhin and Polenov, 1993; Polenov and Chetverukhin, 1993). In adult reptiles, addition of neurons has been reported in the olfactory bulb, throughout the forebrain and diencephalon while no neurogenesis has been reported in the midbrain or hindbrain (Font et al., 2001; Garcia-Verdugo et al., 2002). In birds, adult neurogenesis has first been described in the higher vocal centre (HVC), located in the avian telencephalon (Goldman and Nottebohm, 1983). Other areas in which neurogenesis has been shown includes the striatum, lobus parolfactorius and hippocampus, while the hypothalamus, septum, cerebellum, optic tectum and brain stem appear to be devoid of neurogenesis (Alvarez-Buylla, 1990; Nottebohm, 1985).

In summary, adult neurogenesis in non-mammalian vertebrates is morphologically more widespread compared to mammals, and several brain areas have addition of new neurons. There is some conservation concerning which brain areas have addition of neurons, such as the olfactory bulb, and there are also some phyla specific neurogenic areas like the HVC in birds. The progenitor cells for these neurons are thought to be the RGLs lining the ventricular system of these animals, but genetic fate mapping is needed to show this decisively.

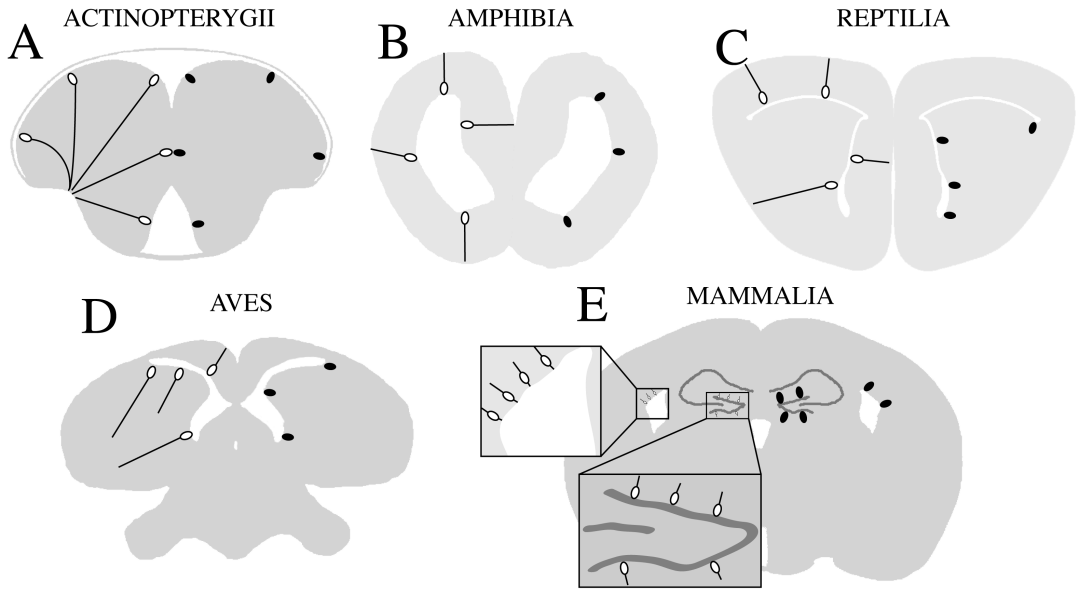

Figure 3) Differences in brain morphology and distribution of proliferation of progenitor cells in five vertebrate classes.
Empty circles on the left hemisphere indicate the location of radial glia-like cell nuclei and lines show the radial projections. Filled circles on the right hemisphere indicate the distribution of proliferating cells. The brains are chosen from following species: **A)** zebrafish, **B)** salamander, **C)** gecko, **D)** atlantic canary and **E)** mouse (note that the section chosen is at the very caudal end of the lateral ventricles). Figures not drawn to scale.

2.1.2 Adult neurogenesis in mammals

2.1.2.1 Dentate gyrus of the Hippocampus

In the adult mammalian brain, neurons are continuously generated in the SGZ of the dentate gyrus and SVZ of the lateral ventricles. The neurons that are generated in the adult mammalian SGZ are dentate granule neurons. These cells are derived from a set of progenitor cells found lining the hilar side of the granule cell layer of the dentate gyrus. The neural progenitors in the SGZ can be divided into three cell types, radial-glial like cells (sometimes denoted as type I cells), intermediate progenitor cells (IPCs) (sometimes called type II cells), and neuroblasts (Kriegstein and Alvarez-Buylla, 2009; Ming and Song, 2011).

The type I cells are thought to be the bona-fide stem cells, and have a radial glia-like morphology and express glial fibrillary acidic protein (GFAP) and nestin (Bonaguidi et al., 2011). Both of these proteins are markers for stem cells in the adult and developing CNS (Fukuda et al., 2003; Lendahl et al., 1990). The RGLs are thought to be mostly quiescent and are therefore usually negative for markers of cell proliferation, such as PCNA and MCM2 (Bonaguidi et al., 2012; Lugert et al., 2010). The RGLs give rise to the IPCs. These cells have low, if any levels of GFAP and nestin, but are highly proliferative. The IPCs start express proneural transcription factors such as Mash 1 (also known as Ascl1) (Castro et al., 2011) and late-stage IPC express doublecortin (Dcx). IPCs give rise to neuroblast that also express Dcx, and are somewhat proliferative. Neuroblasts give rise to immature neurons, which still are Dcx^+ and start to express the pan neuronal marker NeuN. These cells eventually mature into glutamatergic granule cells (or dentate granule cells).

The development from RGLs to a mature and functional granule cells takes around 6-8 weeks (Mongiat and Schinder, 2011; Zhao et al., 2006). The axons of the mature granule cells project through the hilus to the CA3 region of the hippocampus, where they form synaptic connections (Markakis and Gage, 1999; Mongiat and Schinder, 2011). In rodents, it has been estimated that several thousands of neurons are added to the dentate gyrus every day, though a large number of newborn neurons are thought to die within the third week after cell division (Cameron and McKay, 2001; Kempermann et al., 2003). Neurogenesis in the hippocampus changes with age and the number of neurons produced in the rodent dentate gyrus declines in old animals (Kuhn et al., 1996).

2.1.2.2 Subventricular zone of the lateral ventricles

In the lateral wall of the lateral ventricles, stem cells are found beneath a layer of ependymal cells that line the ventricular system. Progeny of these the stem cells migrate rostrally to the olfactory bulb and mature into periglomerular or granule neurons (Kriegstein and Alvarez-Buylla, 2009; Ming and Song, 2011).

The progenitor cells in the lateral wall can be divided into three groups, sometimes denoted as type B, type C and type A cells. Type B cells are considered to be the primary progenitors and are located in the subventricular zone (SVZ) (Doetsch et al., 1999a; Kriegstein and Alvarez-Buylla, 2009). Type B cells have a radial glia-like morphology with a short apical process in contact with the ventricle and a long basal process with end feet touching blood vessels (Ming and Song, 2011; Shen et al., 2008;

Tavazoie et al., 2008). Type B cells, is a population of mostly quiescent cells but when they proliferate they give rise to the transiently amplifying, type C cells (Doetsch et al., 1999b; Morshead et al., 1994). Type C cells are highly proliferative cells that in contrast to type B cells do not have any contact with the ventricle. Type C cells give rise to immature neuroblasts, also called type A cells. Neuroblasts migrate to the olfactory bulb by what is called the rostral migratory stream (RMS). The majority of them differentiate GABAergic granule neurons while a minority differentiate into GABAergic periglomerular cells (Luskin, 1993). One study suggests that there is a small population of glutamatergic short-axon, juxtaglomerular cells being generated in the adult olfactory bulb (Brill et al., 2009). Many of the young neurons undergo apoptosis and of the cells generated and only around 50% remain after 45 days. The neurons that survive the first three months can survive for at least 19 months (Petreanu and Alvarez-Buylla, 2002; Winner et al., 2002). The survival rate of the new born neurons can be artificially increased by exposing the animals to an array of different odours, suggesting that activity of neurons increases survival (Rochefort et al., 2002). But the effect observed appears to last only short-term, because reduction of sensory input causes the number of surviving cells to return to control levels (Rochefort and Lledo, 2005).

2.1.2.3 Neurogenesis in other areas of the adult mammalian brain

Apart from the SGZ of the dentate gyrus in the hippocampus and SVZ of the lateral ventricles, neurogenesis has been reported in other areas of the mammalian brain including hypothalamus, substantia nigra and amygdala (Gould, 2007). While addition of neurons has been reported in the neo-cortex of adult macaques and rodents by some groups (Dayer et al., 2005; Gould et al., 1999), other groups consistently fail to find new neurons in these species and brain areas (Ehninger and Kempermann, 2003; Kornack and Rakic, 2001; Magavi et al., 2000). Neurogenesis of DA neurons in the substantia nigra of the rodent midbrain is also a controversial issue with contradictory results (Frielingsdorf et al., 2004; Lie et al., 2002; Zhao et al., 2003). Differential findings may be due to the use of different methods to neurogenesis in the brain. Examples of these differences are variations in thickness of the sections analysed (for example, using too thick sections with low sampling frequency might lead to $BrdU^+$ neurons not being detected). Also differences in microscopy techniques and different protocols of BrdU administration might be the cause of these discrepancies (Frielingsdorf et al., 2004).

Neurogenesis has also been observed in the adult hypothalamus of adult rodents and adult sheep (Kokoeva et al., 2005; Migaud et al., 2010; Seress, 1985). BrdU pulse chase experiments and genetic fate mapping has shown that tanycytes, located in the ventral hypothalamic ventricular zone, are actively proliferating progenitor cells in the hypothalamus. Apart from being situated in the hypothalamus, tanycytes are highly reminiscent of the stem cells in the SVZ and SGZ, they have a radial glia-like morphology and express neural stem cell markers such as nestin and Sox2 (Lee et al., 2012; Migaud et al., 2010).

It is interesting to note that most of the stem cells described in the adult mammalian brain have a radial glia-like morphology, whether they are found in the SVZ, SGZ or even ventral hypothalamus. This observation suggests that there might be a relationship between cell morphology and cell multipotency, though I am not aware of any research addressing this issue.

2.1.3 Adult neurogenesis in humans

Most studies on mammalian adult neurogenesis are performed on rodents. Identifying genesis of cells in humans is a very difficult thing to do. What is common practice in animal studies, such as administration of nucleotide analogues or virus, is not possible in healthy humans due to possible toxicity, mutagenicity and carcinogenicity of these chemicals. The presence of cycling cells can be studied using immunohistochemistry with antibodies against proteins present in proliferating cells such as PCNA, KI67 or MCM2 on post-mortem brain tissue. This technique has been used, and proliferating cell have been identified in the human SVZ but the final destination of the progeny of these cells cannot be found using this technique (Sanai et al., 2004). BrdU has been administered to cancer patients as a diagnostic tool. This was done to assess the proliferation rate of the cancer cells in the tumour after biopsy. Post-mortem brains from these patients were analysed and $BrdU^+$ neurons were observed in the dentate gyrus of the hippocampus and the olfactory bulb, but no $BrdU^+$ neurons were observed in the cortex (Bhardwaj et al., 2006; Curtis et al., 2007; Eriksson et al., 1998). It should be noted that the presence of adult neurogenesis in the olfactory bulb is a highly controversial issue and other groups find limited addition of neurons to the adult human olfactory bulb (Sanai et al., 2011; Sanai et al., 2004). Also the existence of

a rostral migratory stream in humans is highly debated, with different groups having contradictory results (Curtis et al., 2007; Sanai et al., 2011; Sanai et al., 2004).

A caveat with human BrdU studies is that the subjects are terminally ill and have been treated with drugs to cure the cancer, and the effect that the diseases or medical treatment has on neurogenesis is difficult to elucidate. To overcome this hurdle an ingenious technique has been developed in which the ^{14}C content in the DNA is measured. The half-life of ^{14}C is 5730 years and the atmospheric ^{14}C levels have been very stable throughout for at least centuries. But above ground nuclear bomb testing in the 1950s and 60s led to a huge increase in the atmospheric ^{14}C levels that subsequently decreased exponentially, since the abandonment of these tests. Measuring the ^{14}C content in the DNA of cells has been shown to be a good method to retrospectively determine when various cells in the human body are born (Bergmann et al., 2009; Spalding et al., 2008; Spalding et al., 2005). Using this technique it has been determined that although there is turnover of non-neuronal cells such as astrocytes, there is no addition of neurons to the adult human cortex (Bhardwaj et al., 2006). This ^{14}C dating method has not provided evidence for new neurons being added to the olfactory bulb of adult humans (Bergmann et al., 2012). It has been pointed that although these Swedish subjects did not show signs of olfactory neurogenesis, this phenomenon might be present in people who use their olfaction to a higher degree than ordinary humans such as wine sommeliers and chefs (Macklis, 2012).

2.2 COMPARATIVE ASPECTS OF ADULT NEUROGENESIS IN VERTEBRATES

Interestingly, there seems to be a certain degree of conservation regarding areas of the brain in which neurons are added in the adult animals. For example, the olfactory bulb is a structure preserved in vertebrates ranging from the jawless fish to mammals (Melendez-Ferro et al., 2001). Neurogenesis in the adult olfactory bulb appears to be conserved amongst most studied vertebrates, with a possible exception found in the humans (Adolf et al., 2006; Bergmann et al., 2012; Garcia-Verdugo et al., 1989; Richter and Kranz, 1981; Vellema et al., 2010).

The dentate gyrus of the hippocampus, on the other hand, is a structure only found in mammals (Treves et al., 2008). While birds do have a hippocampus, the homolog for this structure in fish, amphibians and reptiles is thought to be the lateral pallium and the medial pallium (Northcutt, 1995; Vargas et al., 2009). Recruitment of new neurons to the hippocampus or the corresponding structure appears to be conserved in most studied vertebrates (Barnea and Nottebohm, 1994; Kaslin et al., 2008).

Although neurogenesis in the adult hypothalamus has not been extensively studied in mammals compared to the SGZ and the SVZ, there are data indicating addition of neurons in this area (Lee et al., 2012). Neurogenesis in the hypothalamus has also been observed in adult fish (Grandel et al., 2006; Zupanc et al., 2005) and frogs (Polenov and Chetverukhin, 1993) but there are no reports showing it in reptiles or birds (Cao et al., 2002; Kaslin et al., 2008).

2.3 ROLE OF ADULT NEUROGENESIS IN THE VERTEBRATE BRAIN

Why are new neurons added to some parts of the brain but not others? And what is the role of these new neurons? These questions are not only interesting from an evolutionary point of view, but are also important in determining possible diseases caused by alterations in adult neurogenesis.

Although the distribution of proliferating progenitor cells and addition of new neurons varies a great deal between different species, there are some features that many animals have in common. Firstly, addition of new neurons in the olfactory bulb has been observed in fish, amphibians, reptiles and mammals (Mackay-Sim, 2010). The

olfactory bulb is the structure of the brain that relays olfactory information from the nose to the piriform cortex brain (Buck, 1996).

As previously mentioned, if rodents are exposed to different odours, the survival rate of the newly born neurons increases and, if neurogenesis in the SVZ is in turn reduced, the survival rate of the neurons that make it to the olfactory bulb is increased, suggesting an activity-dependency for survival of the olfactory neurons (Rochefort et al., 2002; Rochefort and Lledo, 2005). In a recent study, newborn neurons in the olfactory bulb of adult mice were artificially activated by optogenetics. The authors observed that this experimental setup improved the learning capabilities and enhanced mnemonic capacity (Alonso et al., 2012). It remains to be shown whether the survival rate of newborn neurons in the adult brain can be altered through activation by optogenetics. Ablation of neurogenesis has been shown to affect the structure of the olfactory bulb in mammals (Valley et al., 2009), and continuous neurogenesis is required for the preservation and reorganization of the neural circuitry in the olfactory bulb (Imayoshi et al., 2008).

Neurogenesis in the adult dentate gyrus of the hippocampus is the only brain region in which neurogenesis has clearly been demonstrated in humans (Eriksson et al., 1998). This structure is in mammals associated with memory formation, and addition of neurons in this structure has in mammals been connected to memory formation and learning (Zhao et al., 2008). Changes in neurogenesis in the dentate gyrus have also been implicated with various disorders such as stress and depression (Sahay and Hen, 2007; Snyder et al., 2011). However, it is not clear whether these changes are causal or only correlational to the manifestations seen in animal models of these diseases.

Even though non-mammalian vertebrates do not have a dentate gyrus, many of these animals have a great mnemonic capacity. Migratory birds, for example show remarkable long-term memory, and neurogenesis has been observed in the avian hippocampus (Alvarez-Buylla, 1990; Godard, 1991). The structure that is thought to functionally and cellularly correspond to the hippocampus in fish, amphibians and reptiles is the dorsal and lateral pallium (Rodriguez et al., 2002). Interestingly, neurons are continually added to these structures in many animals, raising the tantalizing possibility that the function of adult neurogenesis in this structure might be conserved between different taxa. Further research needs to be performed to address this possibility.

Neurogenesis in the hypothalamus has been observed in several species of vertebrates including rodents (Kokoeva et al., 2005). If neurogenesis in this area is blocked by means of irradiation, mice show a change in body weight, indicating that addition of neurons to this area is involved in metabolism (Lee et al., 2012). Inhibition of neurogenesis in the hypothalamus in other phyla would give answer if this is an evolutionary conserved mechanism.

In birds, the structure most studied concerning adult neurogenesis is the HVC, a forebrain nucleus in the pallium that controls singing (Hahnloser et al., 2002). Male canaries (*Serinus canaria*) are seasonally breeding songbirds. They are open-ended vocal learners, which means that they modify their song during adulthood. In this species, neurogenesis in the HVC was thought to be involved in the learning and memorizing of new songs, since the rate of neurogenesis is positively related to seasonal differences in song learning (Goldman and Nottebohm, 1983; Kirn et al., 1994). But when researchers looked at birds that do not learn new songs each season, such as the song sparrow (*Melospiza melodia*) they observed the same seasonal pattern (Tramontin and Brenowitz, 1999). It has been proposed that addition of neurons to the HVC can assist the perception of songs, but the role of adult neurogenesis in the avian HVC still remains an elusive issue (Walton et al., 2012).

2.4 NEUROGENESIS AFTER INJURY

Despite the presence of neural stem cells in the adult mammalian brain, mammals are not able to regenerate significantly after injury or disease (Bjorklund and Lindvall, 2000; Illis, 2012). Lower vertebrates, such as fish, amphibians and reptiles on the other hand, have the capacity to functionally repair structures of the central nervous system (Kaslin et al., 2008; Tanaka and Ferretti, 2009). Here I will compare differences and similarities of the reaction to injury of the CNS between animals that are able to regenerate brain structures, and ones that are not, and discuss reasons that may be the cause for this dramatic difference.

2.4.1 Neurogenesis after injury in the non-mammalian brain

2.4.1.1 Fish

Most regenerating animal models react to injury by increasing proliferation of various cell types, both in the parenchyma and in the ventricular zone. To model traumatic brain injury and kill neurons in the zebrafish brain, a stab lesion model has been developed. A stab to the dorsal telencephalon leads to massive cell death, seen by dUTP nick end (TUNEL) labelling. TUNEL is a method used to detect DNA fragmentation and thus cell death. The phenotype of the cells that died after this stab lesion was not examined, and it is likely that both glia and neurons die. A proliferative response, assed by BrdU incorporation and PCNA expression, starts three days after the stab lesion. This increase in proliferation remains higher compared to the control hemisphere for at least 14 days after the injury, and proliferating cells are observed both in the ventricular zone and in the parenchyma. Using BrdU pulse-chase experiments and Cre-loxP-based genetic lineage-tracing techniques, the authors suggest that the newly generated neurons were derived from ventricular RGLs (Kroehne et al., 2011).

2.4.1.2 Reptiles

In reptiles, the neurotoxin 3-Acetylpyridine (3AP) has been used to kill neurons in different kinds of lizards. 12 hours after 3AP was injected into the cortex, neural death was observed. Proliferation was detected using both BrdU and antibody staining against proliferation cell nuclear antigen (PCNA). 1-2 weeks after the injury, an increase in proliferation was observed and it was hypothesised but not shown, that progeny of these cells migrate and differentiate and replace the lost neurons (Font et al., 2001; Font et al., 1991; Romero-Aleman et al., 2004).

2.4.1.3 Birds

As previously mentioned, adult neurogenesis occurs naturally in the HVC in birds (Hahnloser et al., 2002). Experimental ablation of neurons in the HVC by photolysis results in a proliferative response, but the origin of the proliferating cells is not known. The neurons that are normally renewed in the HVC, the projection neurons, can be regenerated, while the neurons that are not normally generated in this area, the HVC-X neurons, do not appear to be regenerated (Scharff et al., 2000). Compensatory proliferation has also been observed in avian hypothalamus, an area where no natural neuronal turnover has been observed. When the hypothalamus is injured, new neurons are generated, but the functionality of these neurons has not yet been addressed (Cao et al., 2002).

2.4.1.4 Amphibians

The first regenerative studies done on the amphibian brain used the technique of simply surgically removing a piece of the brain that one wanted to study. In these early studies, an increase in proliferation was also observed, but the identity of the proliferating cells was not thoroughly investigated (Minelli et al., 1987). Experiments have been done on newts, in which a 70% of the optic tectum is surgically removed. A proliferative response was observed in these animals and after 8 months the tectum was almost fully regenerated, but the origin of the new neurons was not investigated (Okamoto et al., 2007).

Toxin induced neuronal death has also been used in amphibian animal models, for example 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is a toxin that causes Parkinsonian like symptoms in humans and animals. This toxin has previously been administrated to both frogs and salamanders, but the question if the animals regenerated was not asked (Barbeau et al., 1985a; Barbeau et al., 1985b; Langston et al., 1983). Another toxin used to kill DA neurons is animal models is 6-hydroxydopamine (6-OHDA), which is an analogue to catecholamine receptors (Kelly et al., 1975). 6-OHDA has also been injected into the brain of frogs, though without examining if there was a proliferative response to the injury or if the animals regenerated (Endepols et al., 2004). In the red spotted newt, 6-OHDA has been used to kill the DA neurons in the midbrain. 3 days after injection of 6-OHDA, $TUNEL^+$ cells expressing TH were observed and the majority of the DA neurons in the midbrain were lost. Quantification showed reduction of cells expressing other midbrain DA markers,

such as TH and Nurr1 (Parish et al., 2007). The midbrain DA neurons are known to regulate locomotion and in order to examine the functionality of DA cell loss a behaviour assay was developed in which locomotion was quantified after an injection of amphetamine into lesioned and sham-lesioned animals. Lesioned newts showed decreased locomotion compared to control animals.

30 days after 6-OHDA lesion, complete cellular and behavioural recovery was observed. After injury, an increase in ventricular $BrdU^+$ was observed and after a 30-day chase, BrdU/TH double positive cells were identified, indicating that regeneration of midbrain DA neurons was fuelled by neurogenesis (Parish et al., 2007). The observation that the newt can fully regenerate its midbrain DA neurons makes it a unique animal model and allows for further questions to be addressed in this animal model. For example, what is the identity of the progenitor cell that gives rise to new neurons after injury? What are the signals that control initiation and termination of the DA neuron regeneration process? Can the newt regenerate other types of neurons in the midbrain?

2.4.2 Neurogenesis after injury in the mammalian brain

Under normal conditions, addition of neurons in the adult mammalian brain is thought to be restricted to certain well-defined brain areas such as the olfactory bulb and the dentate gyrus of the hippocampus. This does not exclude the possibility that neurogenesis occurs in other areas after injury or during disease. Interestingly, neurogenesis in the neurogenic niches themselves is increased in animals subjected to brain injury and in animal models for various brain diseases. For example, if seizures are induced in rats using the toxin Pilocarpine, the number of generated granule cells is significantly increased (Parent et al., 1997). Cerebral ischemia or stroke can also be modelled in animals by disturbing the blood supply to the brain, which leads to extensive neuronal death in the brain. This treatment has also been reported to increase proliferation of neural progenitors both in the SVZ and the SGZ in the mammalian brain (Arvidsson et al., 2001; Matsuoka et al., 2003; Zhang et al., 2001). Modelling stroke in the rodent brain can also induce proliferation of the normally quiescent ependymal cells found lining the ventricular system that then give rise to neuroblasts (Carlen et al., 2009; Zhang et al., 2007). Intriguingly, some progeny of the injury induced proliferating cells give rise to new neurons in the striatum (Arvidsson et al., 2002). These cells are thought to originate from the SVZ, express Dcx, and migrate into

the striatum. The functionality and electrical properties of these neurons has not been examined and it is thought that the majority of these neurons do not survive (Arvidsson et al., 2002; Parent, 2003).

Neuronal death can be induced in different areas of the brain using a chromophore-targeted neuronal degeneration (Sheen and Macklis, 1995). When this method is used to kill neurons in the rodent cortex, a proliferative response has been observed and new neurons are generated in the injured area. The origin of these new cells is not clear but they appear to be originating from the SVZ (Magavi et al., 2000).

An increase in cell proliferation after injury has also been observed in the rodent midbrain. For example if the DA neurons in the SNc are killed using neurotoxins such as MPTP and 6-OHDA, an increase of proliferation has been observed in both ventricular and parenchymal cells in the midbrain (Aponso et al., 2008; Zhao et al., 2003). It is known that the ventral midbrain harbours cells with multipotent stem cells potential, and if cultured *in vitro* can give rise to cells in all of the neural lineages (Lie et al., 2002; Storch et al., 2004). Despite the presence of multipotent cells in the midbrain, the question if the injury-activated cells give rise to new DA neurons in the midbrain is a controversial issue (Frielingsdorf et al., 2004; Zhao et al., 2003). A non-disputed issue nonetheless, is that mammals, unlike the salamanders, do not regenerate their midbrain DA neurons to a significant degree after injury or disease.

2.4.3 Why are some animals able to regenerate brain tissue while others are not?

The ability to regenerate tissues such as the brain is a feature not found in all vertebrate species, but it is present in some fish, amphibians and reptiles. It is unlikely that these animals have developed the capacity to regenerate the brain independently, since many features of the regeneration process appear in different regeneration models. How come these animals have the capacity to regenerate brain tissue whilst mammals cannot?

At first glance it might appear clearly beneficial for an animal to be able to regenerate a tissue or an organ. But to develop the capacity to regenerate an organ such as the brain or a limb is a complicated matter. Firstly there must be an evolutionary pressure to develop or retain the regenerative capacity. This pressure can be either a direct pressure on regeneration as a trait itself, or an indirect pressure and regeneration has then developed as an epiphenomenon, or as a by-product of another trait (Brockes et al., 2001; Tanaka and Ferretti, 2009).

For regeneration of a specific organ to have developed through direct evolutionary pressure, a certain set of logical criteria need to be met by the animal (Wagner and Misof, 1992).

- The organ in question is liable to damage in the natural habitat of the animal and damage to the organ occurs relatively frequently.

- The animal can survive without a completely functional organ.

- The animal's fitness increases if the organ in question regenerates.

- The time it takes for the organ to regenerate cannot be too long relative to the lifespan of the organism.

Using the newt limb regeneration as an example, attacks from fish, crayfish and other newts are prevalent in the newt's natural habitat, and lead to loss of limbs (Marion and Hay, 2011; Pfennig and Collins, 1993). Since the newts live mostly in water and are very competent swimmers even when they have lost a limb, they have no problem to survive without a limb for the 75 days it takes for them to regenerate a new

one. But newts do use their limbs for reproduction. These animals reproduce through a form of pseudocopulation called amplexus, in which the male newt grasp the female with his hind limbs (Able, 1999). It is possible that newts that do not possess a full set of limbs are not able to reproduce. This has however not been extensively studied in newts. A newt that has all its limbs will nevertheless have fitness compared to newts lacking limbs.

Interestingly, frogs are able to completely regenerate their limbs as aquatic tadpoles, but this phenomenon is lost after metamorphosis (Kurabuchi, 1990; Suzuki et al., 2006). As adults, terrestrial frogs are heavily reliant on their limbs for locomotion (Astley and Roberts, 2012; Lutz and Rome, 1994). If they loose a limb they are therefore not likely to survive for long, and definitely not long enough to regenerate a new limb. It could be argued that since these animals do not survive long enough to regenerate, the capacity to regenerate cannot be evolved or retained in these animals. In some aquatic frogs, such as the African clawed frog (*Xenopus laevis*), amputation of limb results in the generation of a muscle-deficient, cartilagenous spike (Yakushiji et al., 2009). This spike enhances the male frogs ability to form amplexus with females and thus enhances the animals' ability to reproduce (Yakushiji et al., 2009), demonstrating that even partial regeneration can be beneficial for an animal.

Unlike limb regeneration, the capacity to regenerate brain tissue does not only exist in newts, but has been observed in fish, other amphibians and reptiles. It has been argued that the capacity of these animals to regenerate brain tissue after injury has evolved as an epiphenomenon or by-product of another trait (Kaslin et al., 2008; Tanaka and Ferretti, 2009). According to this theory, the constant growth of certain brain parts, primarily sensory parts of the brain, has been selected for to the benefit of the animals. Regeneration of these brain parts is possible since the functional requirements for growth are already in place in these areas, and the environment is already permissive for cell growth and neural integration into the existing neuronal networks (Ferretti, 2011). Experiments to test this hypothesis should examine if it is possible for some animals to regenerate areas in the brain where neurogenesis does not take place under normal conditions. In **Paper I** of this thesis we map the presence of proliferating cells in the adult newt brain, and examine if areas devoid of proliferating are able to regenerate.

It is not clear whether a frequent and sub-lethal injury to the brain has put evolutionary pressure to develop brain regeneration as a trait. As previously mentioned, red spotted newts spend parts of winter in ice-covered ponds, an environment which is

known to become hypoxic (Berner and Puckett, 2010; Tattersall and Boutilier, 1997). In **Paper III** of this thesis we test if the newt is able to survive hypoxia under controlled conditions, and if this treatment leads to brain damage.

3 NEUROTRANSMITTERS AND HOW THEY CONTROL STEM CELLS

3.1 NEUROTRANSMITTERS

3.1.1 Description

The primary way for neurons to communicate with each other is through the synapse, a specialized junction where chemicals are released by the presynaptic neuron and react with receptors on the postsynaptic neuron. These chemicals are called neurotransmitters. There are two major groups of neurotransmitters in the central nervous system, amino acids and amines. Examples of amino acids include glutamate, γ-aminobutyric acid (GABA) and glycine. While dopamine, and norepinephrine, epinephrine and serotonin (5-HT) are the major amine neurotransmitter. Other neurotransmitters include acetylcholine (ACh), which is an ester of acetic acid and choline, and small peptides such as substance P and somatostatin.

The same neurotransmitter can bind to an array of different receptors and thus the effect of the neurotransmitter varies depending on what combination of receptors is expressed by the receiving cell. Importantly, the release of neurotransmitters is not only confined to the synapse, but also occurs directly to non-synaptic extracellular space (Blankenship and Feller, 2010). In the adult hippocampus GABA and glutamate is released in extra synaptic areas (Brickley and Mody, 2012; Rusakov and Kullmann, 1998) and in the midbrain, dopamine is released by dendrites belonging to the DA neurons located in the substantia nigra and ventral tegmental area (Beckstead et al., 2004; Bjorklund and Lindvall, 1975; Geffen et al., 1976).

Neurotransmitter signalling is not only an inter-neuron phenomenon, but can also take place between neurons and other cell types such as glial cells and neural progenitor cells. Neurotransmitters have been shown to regulate the proliferation and maturation of progenitor cells into neurons showing that neural activity and experience can control the behaviour of progenitor cells. Neurotransmitters could therefore constitute an important signalling mechanism during development and regeneration. In **Paper II** of this these we examine the role of the neurotransmitter dopamine in controlling regeneration of DA neurons.

3.1.2 Neurotransmitter control of neurogenesis and stem cells

Neurotransmitter control of adult neurogenesis gives a fascinating link between animal behaviour and the formation of new neurons. The process of neurogenesis is tightly regulated by behaviour. Voluntary running, for example increases proliferation of neural progenitors, while animals that are subjected to social isolation show decreased neurogenesis in the hippocampus (Ibi et al., 2008; van Praag et al., 1999). It has been hypothesized that these effects are due to changes in activity of neurons in the neurogenic areas and subsequent alterations in the levels of neurotransmitters, but as of today this has not been directly demonstrated.

Neurotransmitters do not only function as signalling molecules at the synapse, but are also used in the body to control stem cell proliferation and neurogenesis. One example of a neurotransmitter that controls stem cells is GABA. GABA has an inhibitory effect on mature neurons while it excites immature neurons during development and in an adult context (Cancedda et al., 2007; Toni and Sultan, 2011). In development and in the adult animal, GABA signalling inhibits proliferation of neural stem cells through the ionotropic $GABA_A$ receptor (Andang et al., 2008; Liu et al., 2005; Tozuka et al., 2005). A recent study using optogenetics has shown that RGLs in the SGZ react to GABA that is released by the Parvalbumin expressing interneurons. Activation of these interneurons, but not somatostatin or vasoactive intestinal polypeptide expressing interneurons, induced quiescence of the RGLs (Song et al., 2012). Also glutamate has been shown to control proliferation and differentiation of stem cells in the adult brain. Treatment with an NMDA receptor agonist reduces proliferation of neural progenitor cells in the SGZ (Cameron et al., 1995) but it is not clear if this effect is cell autonomous, or occurs via regulation of other cell types. Specific deletion of NMDA receptors in neural progenitor cells reduces the survival rate of immature neurons (Tashiro et al., 2006).

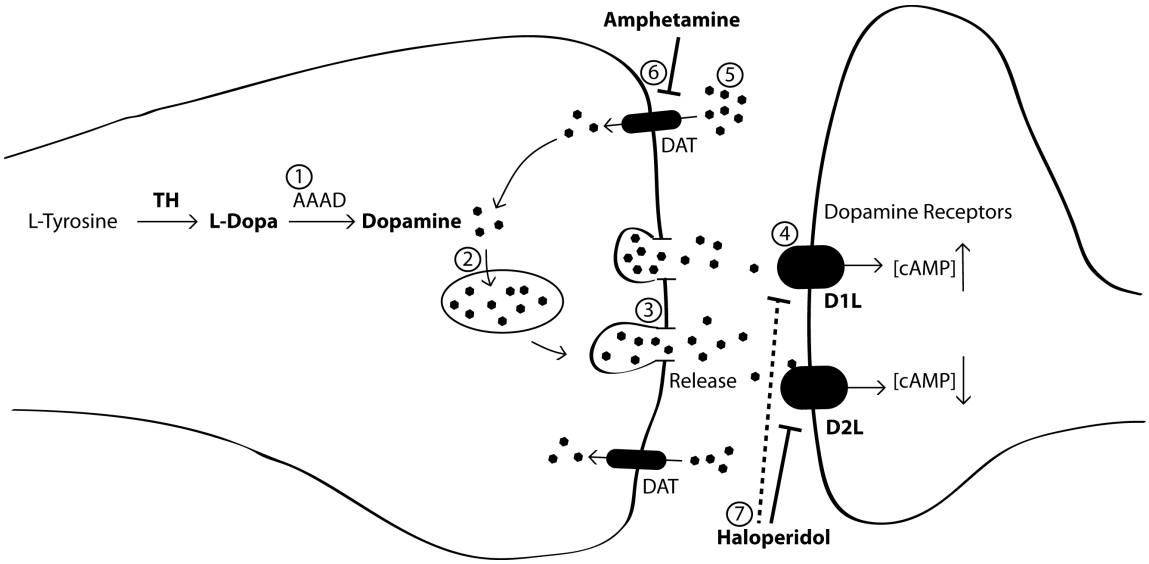

Figure 4) Dopamine synthesis, turnover and transduction in a classical synapse.
1) Dopamine is synthesized in the presynaptic cells from L-tyrosine and L-dopa.
2) Dopamine is loaded into vesicles that are 3) emptied into the synaptic cleft upon depolarization. 4) Dopamine binds to D1L or D2L dopamine receptors that have opposing effects on the postsynaptic cells intracellular concentration of cAMP.
5) Dopamine is transported into cells by dopamine transporter (DAT).
6) Amphetamine inhibits the DAT, thereby increasing the concentration of dopamine in the synaptic cleft. 7) Dopamine receptor antagonist Haloperidol inhibits both D1L and D2L dopamine receptors, but has significantly higher affinity for D2L dopamine receptors.

3.2 DOPAMINE

3.2.1 Dopamine and its receptors

Dopamine was discovered to be a neurotransmitter in 1958 by Arvid Carlson and is now known to be a major catecholamine neurotransmitter in the metazoan nervous system (Carlsson et al., 1958; Missale et al., 1998). Dopamine is involved in controlling a variety of functions including locomotion, cognition, reward and neuroendocrine function. Dopamine is synthesised from the amino acid tyrosine, which is converted by tyrosine hydroxylase (TH) into $_{L}$-3,4-dihydroxyphenylalanine (L-dopa). L-dopa is in turn transformed into dopamine, a reaction catalysed by aromatic $_{L}$-amino acid decarboxylase (AAAD). TH is the rate limiting enzyme in dopamine synthesis and is commonly used as a marker for DA neurons (Elsworth and Roth, 1997).

Dopamine receptors are metabotropic and heterotrimeric G protein coupled receptors and are classified as either D1-like (D1L) or D2-like (D2L). The D1L receptor family consists of dopamine receptors D1 and D5. When dopamine binds to

the D1L receptors, the $G_{\alpha s}$ subunit stimulates the activity of adenylate cyclase, this leads to increased production of cAMP (Cyclic adenosine monophosphate).

The D2L receptor family consists of dopamine receptors D2, D3 and D4. Activation of the D2L receptors leads to inhibition of adenylate cyclase, through the $G_{\alpha i}$ signalling. Activation of D2-like receptors results in decreased production of cAMP (Figure 4) (Emilien et al., 1999; Missale et al., 1998). Hence dopamine signalling through D1L and D2L receptors has opposing effects on the postsynaptic cell.

The effect that dopamine has on the different cells is also context dependent. Activation of different receptors results in activation of distinct intracellular signalling pathways. To complicate things further, dopamine receptors also exist in heterodimers. These heterodimeric complexes can be formed by either two different kinds of dopamine receptors or one dopamine receptor and another type of receptor, such as the ionotropic NMDA receptor (Ferre et al., 2009; Missale et al., 2010; Salter, 2003).

3.2.2 Diseases of Dopamine

Deficits and alterations in dopamine signalling are involved in diseases such Parkinson's disease (PD) (Dawson and Dawson, 2003), schizophrenia (Knable and Weinberger, 1997), drug addiction (Koob and Le Moal, 2001) and attention-deficit hyperactivity disorder (ADHD) (Faraone et al., 2005). Treatment of these diseases often involves direct or indirect modulation of dopamine signalling. Examples of drugs used in the treatment of some of these diseases are amphetamine treating ADHD and dopamine receptor antagonist such as haloperidol in treatment against schizophrenia. Amphetamine increases the concentration of dopamine by inhibiting the dopamine transporter (DAT) (Giros et al., 1996; Wigal, 2009) and haloperidol is a dopamine receptor antagonist with a high binding affinity for D2L receptors (Missale et al., 1998). Care has to be taken when modulating the DA system over time, since this treatment might lead to chronic complications. Long term haloperidol treatment for example can lead to a condition called tardive dyskinesia, which is a movement disorder characterised by involuntary movement of the face and extremities (Margolese et al., 2005). How dopamine receptor antagonists cause tardive dyskinesia is not known (Alabed et al., 2011; Tammenmaa et al., 2002).

3.2.2.1 Parkinson's disease

Parkinson's disease (PD) was first described in 1817 by British physician James Parkinson in his "Essay on the Shaking Palsy" (Parkinson, 2002). In this paper Parkinson described a devastating disease with primary symptoms of tremors and general difficulties in movement. We now know that Parkinson's disease (PD) is a slowly progressing neurodegenerative disease that affects ~1-2 % of individuals over the age of 60. The primary symptoms of PD are inability to move (akinesia), slowness of movement (bradykinesia), tremor and postural instability (Olanow et al., 2009b).

The major cause of these symptoms is thought to be loss of DA neurons in the ventral midbrain, more specifically in the substantia nigra pars compacta (SNc). The DA neurons in the SNc project to the striatum, and death of these neurons leads to a decrease in the concentration of dopamine in the striatum (Olanow et al., 2009b). In addition to the death of midbrain DA cells the pathology also consists of the formation of α-synuclein containing Lewy bodies in the SNc and other areas (Kosaka et al., 1976; Spillantini et al., 1997).

The etiology of PD is not fully understood. Both genetic and environmental factors have been implicated in the disease. Several genes are known to cause familiar and sporadic PD, including the parkin gene and α-synuclein gene (Kitada et al., 1998; Singleton et al., 2003). Pesticides and other toxins have also been shown to cause PD-like symptoms in animal models and humans (Betarbet et al., 2000; Langston et al., 1983).

Oxidative stress has been implicated as a cause for PD, and post mortem studies of PD patients have shown increase in oxidative damage and mitochondrial impairment (Dexter et al., 1989). DA neurons in the SNc are known to be autonomous pacemaking cells, constantly releasing dopamine into the striatum (Guzman et al., 2009). It has been suggested that this pacemaking activity leads to increased oxidative stress in these neurons, making them more susceptible to toxin induced mitochondrial oxidative damage compared to other neurons (Guzman et al., 2010).

3.2.2.2 Treatment of Parkinson's disease

There is currently no cure for PD, but it is possible to alleviate the symptoms at least for some time. Current treatments usually aim to increase the levels of dopamine signalling in the brain by administering dopamine precursor L-dopa or dopamine receptor agonists such as bromocriptine (van Hilten et al., 2007).

Since L-dopa crosses the blood-brain barrier (BBB) it can be administered orally and it is usually given together with a carbidopa, an inhibitor of AAAD that does not cross the BBB, thus inhibiting peripheral metabolism of L-dopa. Treating PD patients with L-dopa or dopamine agonists in early- and middle-stage of PD lessens the symptoms of the disease but the effect of treatment decreases with time (Marsden and Parkes, 1977). Chronic, long-term treatment with these substances is also associated with motor complications such as dyskinesia, motor fluctuations and physiological problems (Weiner and Reich, 2008).

A possible way to cure PD and other degenerative diseases of the central nervous system (CNS) could be to replace the lost cells in the brain, either by activating endogenous stem cells or transplanting stem cells after manipulating them *in vitro*.

Grafts of embryonic midbrain tissue have been transplanted into the striatum of PD patients in an attempt to restore the dopamine transmission (Lindvall et al., 1990; Olanow et al., 2003). Some patients in these trials experienced symptomatic relief and shown improved motorics after 16 years post transplantation (Li et al., 2008). But results vary using embryonic tissue and this method is not without ethical complications (Olanow et al., 2009a).

3.2.2.3 Animal models of Parkinson's disease

Animal models of PD, mimicking the loss of midbrain DA neurons, are usually obtained by administering neurotoxins, such as aforementioned 6-OHDA or MPTP, which kill the DA neurons. Genetic manipulations mutating genes that are involved in the specification and survival of DA neurons have also been used to create PD-like symptoms in rodents (Chan et al., 1997; Deumens et al., 2002; Kittappa et al., 2007).

3.2.3 Dopamine control of neurogenesis developing mammalian brain

In the developing rodent brain, dopamine has been shown to modulate various aspects of neurogenesis and different progenitor cells express various sets of dopamine receptors. D2-like dopamine receptors have been shown to be expressed in the proliferating cells in the ventricular zones during development, and modulation of dopamine signalling affects neurogenesis and neural specification in many brain areas including the lateral ganglionic eminences (LGE) and the ventral midbrain (Diaz et al., 1997; Kim et al., 2006; Ohtani et al., 2003). In the developing LGE, activation of D1-like dopamine receptors has been shown to decrease proliferation of DA progenitors, while activation of D2-like dopamine receptors had the opposite effect (Ohtani et al., 2003). Interestingly, the same authors found that the general effect of dopamine on primary cultures from the LGE, was an inhibition of proliferation and *in vivo* administration of L-dopa reduced the number of proliferating LGE progenitor cells (Ohtani et al., 2003). These experiments illustrate the complex nature of dopamine signalling on neural precursors.

In the developing ventral midbrain, dopamine signalling has been shown to regulate neurogenesis and maturation of neurons. The DA neurons in the substantia nigra pars compacta (SNc) originate from the midbrain floor plate radial glial cells (Bonilla et al., 2008; Ono et al., 2007). DA progenitor cells express markers such as Nurr1, Lmx1a, and Msx1, which have been shown to be important transcription modulators in the development of ventral midbrain DA neurons (Andersson et al., 2006; Arenas, 2005; Wallen and Perlmann, 2003). Mice lacking the D1 or D2 dopamine receptors have reduced number of SNc DA neurons, but the mechanism of the dopamine-mediated effect on neurogenesis is not clear (Kim et al., 2006; Parish et al., 2001).

It has been suggested that dopamine signalling is involved in differentiation of DA progenitors by activating Nurr1, a fate-determining transcription factor for midbrain DA neurons. Nurr1 is activated by D2R signalling through ERK signalling and *in vitro* experiments showed that treating primary mesencephalic cultures with D2R agonists increased the number of TH^+ cells. The effect that D2R agonist treatment had on proliferation and survival of newborn neurons was not examined (Kim et al., 2006). To determine if modulation of dopamine signalling has an effect on ventral midbrain DA progenitor proliferation, dopamine receptor knockout animals need to be

analysed with high temporal resolution and experiments need to be performed together with different agonist/antagonist treatments.

3.2.4 Dopamine control of neurogenesis in the adult mammalian brain

In adult animals, dopamine signalling has been shown to modulate neurogenesis. In the SGZ of the hippocampus of the Mongolian gerbil (*Meriones unguiculatus*), dopamine receptor antagonist haloperidol, increases progenitor proliferation (Dawirs et al., 1998). Contrary to this, haloperidol treatment does not affect the proliferation in the SGZ of rats and mice (Halim et al., 2004; Kippin et al., 2005). This discrepancy is possibly due to different experimental paradigms. In the gerbil experiments haloperidol was administered during 24 hours, while the rats and mice were treated for at least three weeks.

In the SVZ of adult rodents, dopamine signalling is known to affect different aspects of neurogenesis and progenitor proliferation. The dopamine itself originates from DA afferents from SNc DA neurons, that arrive at the SVZ during development (Freundlieb et al., 2006; Hoglinger et al., 2004). Progenitor cells in the SVZ express an array of different dopamine receptors, though there remains some controversy as to expression pattern of the various dopamine receptors on the different progenitor cells and the effect that dopamine signalling has on neurogenesis (Hoglinger et al., 2004; Kippin et al., 2005).

D2-like dopamine receptors have been reported to be expressed in the transient amplifying cells (type C cells), and the neuroblasts. While D1-like dopamine receptors have only been observed in the type A cells (Hoglinger et al., 2004; Kippin et al., 2005; Platel et al., 2010). It has been proposed that dopamine stimulates proliferation of type A and/or type C cell progenitors in the SVZ and this effect is transmitted through D2L receptors (Coronas et al., 2004; Hoglinger et al., 2004; Platel et al., 2010). Evidence for this view comes from experiments in which the levels of dopamine in the SVZ were artificially reduced either pharmacologically or by killing the SNc DA neurons using specific toxins such as 6-OHDA or MPTP (Baker et al., 2004; Hoglinger et al., 2004; L'Episcopo et al., 2012). A contradictory study shows that blocking dopamine receptor signalling leads to increased proliferation of type B cells. (Kippin et al., 2005). This was shown by treating mice with dopamine receptor antagonist haloperidol, which results in an increase in proliferating cells in the SVZ and increased neurogenesis in the olfactory bulb. The authors also observed an increase in BrdU label retaining cells after

a long-term pulse chase experiment. BrdU label retaining cells are usually used as a marker for the slowly proliferating, type B cells (Doetsch et al., 1999a; Hsu and Fuchs, 2012; Morshead et al., 1994). *In vitro* experiments in the same study showed that treating cells with dopamine or dopamine receptor 2 agonists, inhibited neurosphere formation, this is an assay commonly used for a quantitating proliferation of neural stem cells (Reynolds and Weiss, 1996; Singec et al., 2006). Both the stimulating effect of haloperidol observed *in vivo*, and the inhibitory effects of agonists seen *in vitro* were not observed when using D2-receptor knock-out animals, indicating that the dopamine D2 receptor is involved in mediating this process (Kippin et al., 2005).

3.2.5 Mechanisms of dopamine control of neurogenesis in the adult and developing mammalian brain

The downstream mechanism with which dopamine regulates the cell cycle has not been thoroughly investigated. It has been hypothesised that dopamine stimulates release of epidermal growth factor (EGF) (O'Keeffe et al., 2009). EGF is a factor that is required, together with fibroblast growth factor (FGF) for long-term survival of *in vitro* cultures of neural progenitor cells (Ming and Song, 2005; Reynolds and Weiss, 1992). EGF is also an important factor for *in vivo* proliferation of neural progenitors. Infusion of EGF has been shown to increase cell proliferation in the SVZ, and mice lacking TGFα, an EGF receptor ligand, show reduced proliferation of SVZ progenitor cells (Kuhn et al., 1997; Tropepe et al., 1997).

Dopamine modulation of adult neurogenesis has also been suggested to be dependent on ciliary neurotrophic factor (CNTF) (Yang et al., 2008). CNTF is known to increase survival of neurons and intra-cortical injection of this factor into adult mice induces an increase in proliferation of the SVZ progenitor cells (Arakawa et al., 1990; Emsley and Hagg, 2003). Adult animals lacking CNTF have reduced amount of proliferating progenitor cells in the SVZ and while proliferation in control animals is increased when the animals are treated with the D2R agonist quinpirole, this effect is not observed in heterozygote and homozygote knockout animals.

It is important to point out that most of these studies are done on a population basis, and it is probable that the effect of dopamine varies depending on the maturation and location of the progenitor cell. Cells within the same population are known to express different levels of D1-like or D2-like receptors, as for example observed in oligodendrocyte precursors (Bongarzone et al., 1998). It is possible that more mature

cells have different receptor expression compared with more undifferentiated cells and thus the effect to dopamine would vary. It is also important to point out that both dopamine D1L and D2L receptors can be expressed on the same cell and in addition these receptors can exist as heterodimers. Thus modulating signalling through a specific dopamine receptor does not prove that this is the sole receptor involved in the process. To elucidate which cells react to the dopamine signalling in specific ways, laser dissection of the different progenitor subtypes could be performed based on morphology or genetic reporter. Single cell RNA sequencing or mass spectrometry on dissected tissue would enable one to detect possible differences in receptor expression on different progenitor subtypes (Rugg-Gunn et al., 2012; Tang et al., 2009). Also knock down of the receptor subtypes in a cell-specific manner could show the receptors involved.

In summary, dopamine is known to affect many aspects of neurogenesis in both the adult and developing brain. But the downstream mechanisms and the identity of the neurons that controls these events are yet to be discovered. In **Paper III** of this thesis we examine how dopamine is involved in the process of regeneration in the adult newt brain and discover an until now unknown mechanism, in which dopamine controls the proliferation of DA progenitor cells.

4 PRESENT INVESTIGATION

4.1 AIMS

The overall aim of this thesis is to elucidate mechanisms of brain regeneration in the red spotted newt.

The specific aims of the different studies are:

Paper I:
Map the distribution of proliferating cells in the uninjured newt brain and investigate what happens to the progeny of these cells, determine which cells give rise to neurons after DA neuron ablation in the newt brain identify genes that are activated during the regeneration process.

Paper II:
Investigate how dopamine is involved in the regeneration process of DA neurons in the adult newt.

Paper III:
Examine if newts are able to survive hypoxic conditions and determine subsequent responses in the brain.

4.2 PAPER I

4.2.1 Results

It has been suggested that the reason why some non-mammalian vertebrates are able to regenerate their brain is due to the presence of constantly proliferating cells throughout the brain of these animals (Ferretti, 2011; Kaslin et al., 2008). Upon brain damage, progeny of these cells are thought to migrate and repopulate the injured tissue. In **Paper I**, we aimed to map the distribution of proliferating cells in the adult newt brain and subsequently examine if there is a correlation between the areas that can be regenerated and the existence of proliferating cells.

Using immunohistochemistry we found that the majority of the cells positive for proliferation markers such as PCNA and MCM2, line the walls of the lateral ventricles of the forebrain and the third ventricle of the diencephalon. The majority of those cells express the RGL marker GFAP and have a RGL- morphology. When examining the midbrain and hindbrain for cell proliferation, we found that the ventricular walls of these areas are quiescent and no detectable neurogenesis occurs in these brain areas.

Using the nucleotide analogue BrdU to spatially trace the progeny of proliferating cells, we find that a subpopulation of $BrdU^+$ cells migrate rostrally to the olfactory bulb, others migrate radially away from the ventricles into the pallium. Interestingly, we found that some of the $BrdU^+$ are positive for the pan-neuronal marker NeuN, indicating that they have differentiated into neurons, both in the olfactory bulb and in the pallium.

Next we intended to study how midbrain RGLs respond to 6-OHDA lesion.

To examine this, we used both BrdU pulse chase experiments and an *in vivo* electroporation technique in which a construct coding for yellow fluorescent protein (YFP) was transfected into the ventricular cells by *in vivo* electroporation. We observed that while some ventricular cells became activated upon sham lesion, the majority of these cells remain proximal to the ventricle. After 6-OHDA lesion, we found a significant increase in the number of ventricular derived cells that have migrated away from the ventricle and differentiated into neurons. These result show that 6-OHDA-induced ablation of DA neurons stimulates activation, migration and differentiation of the otherwise quiescent ventricular progenitor cells.

To address whether $GFAP^+$ RGLs give rise to TH^+ neurons after lesion, we electroporated the midbrain ventricular wall with a construct encoding GFP under the control of the GFAP promoter. Four days after electroporation, animals were lesioned

with 6-OHDA. After 2 weeks, GFP/TH double positive cells were observed in the regenerating newt midbrain following 6-OHDA lesion, while no such cells were observed in sham lesioned control animals. These results show that the $GFAP^+$ RGLs are progenitor cells in the newt brain, and these cells give rise to neurons after injury. Next we performed a gene expression analysis during regeneration with the aim to identify possible factors involved in controlling neurogenesis. For this, ventricular cells were laser micro-dissected from lesioned and sham lesioned newt brains and RNA was isolated from these cells. The RNA was subsequently applied to oligonucleotide microarrays derived from salamander expressed sequence tag and complementary DNA databases. We found that 739 genes were upregulated during regeneration, while 324 were downregulated. Of these genes 14 genes were then validated by quantitative real-time polymerase chain reactions.

One of the genes identified to be upregulated in the microarray was sonic hedgehog (Shh), a morphogen that is important in the patterning of the developing neural tube (Dessaud et al., 2008). Our finding was verified by *in situ* hybridization, in which Shh expression was found to be increased after lesion in the ventral part of the third ventricle. To examine the role of Shh during regeneration, Shh signalling was inhibited by the administration of cyclopamine, a potent Shh signal inhibitor. When cyclopamine was administered after injury, regeneration of TH^+ neurons was inhibited, showing that Shh is an important molecule in the regeneration process of the newt brain.

4.2.2 Future experiments and discussion

Though adult neurogenesis in the newt forebrain was observed, the identity of the nerurons generated in the newt brain remains to be determined. This could be done by performing previously mentioned BrdU pulse-chase experiments, in combination with immunohistochemistry using specific markers against neural subtypes. These experiments would also reveal if the type of neurons generated in different brain areas, glutamatergic neurons in the hippocampus and GABAergic neurons in the olfactory bulb, are conserved between amphibians and mammals.

Using oligonucleotide microarrays we identified Shh and many other possible candidates that could be important in the regeneration process. We found that interfering with hedgehog signalling inhibits regeneration, but does not reduce proliferation of the ventricular progenitor cells. It is thus possible that Shh is involved

in either the differentiation process or survival of newborn neurons. Future experiments should test this by assessing cell death in cyclopamine treated animals compared to controls and examine the identity of the cells generated when hedgehog signalling is blocked.

To study the role of the other candidates, expression changes firstly need to be verified by immunohistochemistry or in situ hybridization. Secondly the functionality of these molecules needs to be manipulated during regeneration, which requires the development of genetic manipulation techniques in newts. Since there are no transgenic red spotted newts available and the process of making transgenic newts must be very cumbersome considering that the animals become sexually mature at 1-2 years of age, other methods need to be adopted. Possible methods include silencing transcription through the use of either small interfering RNA (siRNA) or morpholinos. Currently, these techniques have not been optimized fore the use in newt brains, and energy needs to be placed on developing techniques to manipulate gene expression in the newt.

As previously mentioned, neurogenesis in the olfactory bulb and hippocampus (or corresponding brain area) is a phenomenon conserved in many vertebrate species. A finding of **Paper I** is that neurons are also added to the adult newt olfactory bulb and the hippocampal progenitor, the pallium. Future experiments could also examine the functional role of neurogenesis in these areas in the newt. Is the role of neurogenesis in the newt olfactory bulb linked to olfaction? This could be tested by altering odorant stimulus for the animals and examine the rate of neurogenesis in this brain area. Experiments that have previously shown to alter hippocampal proliferation in rodents could be repeated in newts to examine possible regulatory similarities or differences in the mechanism controlling neurogenesis. One way to test this would be to expose the animal to social isolation or vary the environment that the newts live in. These experiments would reveal if mechanisms controlling neurogenesis in the vertebrate brain are conserved between amphibians and mammals.

4.3 PAPER II

4.3.1 Results

As described in section 3 in this thesis, neurotransmitters have been shown to control various aspects of stem cell behaviour, including proliferation, fate choice and differentiation. In **Paper II** we wanted to examine if the neurotransmitter dopamine controls the niche of neural progenitor cells and hence influences regeneration of DA neurons in the adult newt brain.

To increase the concentration of dopamine in the newt brain we treated the regenerating animals with the dopamine precursor L-dopa. We found the L-dopa treatment reduced the number of TH^+ and $Nurr1^+$ neurons in the newt midbrain and inhibited behavioural recovery. To examine if this phenomenon was specific for DA regeneration we developed a technique to kill cholinergic neurons in the newt brain. When the neurotoxin ethylcholine aziridinium (AF64A) was injected into the midbrain, this killed about 50% of the cells expressing cholinergic neuron marker. Regeneration of these cells was gradual and after 45 days the lesioned animals had almost completely restored the numbers of cholinergic neurons in their brain. Interestingly, administration of L-dopa did not affect regeneration of cholinergic neurons, suggesting that the effect of L-dopa is specific for DA regeneration.

Co-staining for PCNA and GFAP showed that L-dopa treatment reduced the number of proliferating RGLs in the midbrain of 6-OHDA lesioned animals, while L-dopa treatment did not affect progenitor proliferation in AF64A lesioned animals. Further, addition of dopamine to primary cultures of newt neuronal progenitor cells led to reduced incorporation of BrdU in $GFAP^+$ cells, indicating that dopamine itself has an inhibitory effect on RGL proliferation.

Using immunohistochemistry, we found that the RGLs express D2 dopamine receptors. To examine if the effect of L-dopa is mediated through dopamine receptor signalling, we co-administered animals with L-dopa and the potent dopamine receptor antagonist haloperidol. Haloperidol treatment rescued the inhibitory effect of L-dopa on regeneration and RGLs proliferation. These results suggest that dopamine acts on dopamine receptors on the RGLs.

To test whether antagonizing dopamine receptors is sufficient to induce a proliferative response in newt midbrain, we treated uninjured animals with haloperidol. This treatment led to increased proliferation of the RGLs and resulted in an increased number of TH^+ neurons. To examine the origin of the neurons, we expressed YFP in

the ventricular RGLs through electroporation. 24 hours after electroporation YFP expression was restricted to ventricular RGLs. After haloperidol treatment, TH^+ YFP^+ cells were observed in the uninjured newt midbrain, while no such cells were observed in vehicle treated controls. These results show that antagonising dopamine signalling is sufficient to activate quiescent RGLs, and progeny of these cell subsequently differentiate into TH^+ neurons in the newt midbrain.

4.3.2 Future experiments and discussion

It remains to be seen if the neurons generated after haloperidol treatment integrate in the existing networks. It is also not clear whether these neurons or the neurons generated after 6-OHDA lesion, project to the striatum. To test this, retrograde tracing of axons in the striatum using fluorogold could be combined with previously mentioned electroporation paradigms. If YFP, fluorogold and TH triple positive cells are observed after haloperidol treatment or 6-OHDA lesion, this would show that cells originating from the ventricular wall have differentiated into neurons and project to the striatum.

It has also not been conclusively shown that dopamine acts on the RGLs themselves. It is possible that the dopamine signals on neighbouring neurons, that in turn signal on the RGLs. To examine this, different dopamine receptors would need to be specifically silenced in RGLs by siRNA or morpholinos. As previously described, these techniques have not yet been developed for the newt.

As discussed in section 3.2.5, there are numerous hypotheses concerning dopamine control of progenitor cell proliferation including EGF signalling and signalling through CNTF. Because of the lack of efficient in vitro techniques and genomic information, the newt may not be the ideal model for the identification of downstream mechanisms for dopamine control of progenitor proliferation, and these experiments would be more feasible to do in other model systems. However it is possible that the mechanisms are not the same in different species, so any signalling pathway identified needs to be verified in the newt.

Finally, it would be very interesting to examine if dopamine has the same inhibitory effect on progenitor cell proliferation in the developing mammalian midbrain. Some simple experiments could be done to answer this. Firstly, the expression pattern of the different dopamine receptors needs to be analysed in the developing mammalian midbrain. If receptors are detected, signalling of these receptors could be pharmacologically manipulated by treatment of various dopamine receptor

agonists and antagonists during different stages of development. Since knockout mice of various dopamine receptors exist, embryos of these animals at different stages of development could be analysed to identify potential changes in progenitor cell proliferation (Drago et al., 1994; Parish et al., 2001).

Finally, the results from this project raise the tantalizing possibility that stem cells can be activated in the brain by administration of a drug. It would be interesting to examine if antagonising dopamine signalling induces neurogenesis in the adult mammalian midbrain. This would have implication for regenerative therapies against neurodegenerative disorders, and could possibly explain the etiology of some neurological disorders such as tardive dyskinesia, which are caused by long-term use of antipsychotic drugs.

4.4 PAPER III

4.4.1 Results

It has been proposed that the capacity of some animals to regenerate brain tissue is linked to continuous neurogenesis in these brain areas. In **paper I** we demonstrate that the newt is able to regenerate brain areas, which under normal circumstances are devoid of proliferating cells, showing that there is no link between regeneration and continuous neurogenesis in the newt brain.

So how come some animals have retained the capacity to regenerate brain tissue while others cannot? As explained on section 2.4.3, if the trait of regenerating a specific organ has been directly selected for, this organ must have been subjected to damage relatively frequently. It is known that the newt limb is susceptible to damage from predators and other newts, and it has been shown that the newt lens is prone to damage from a parasite infection (Marion and Hay, 2011; Okada, 2004). But possible causes of injury to the brain of the newt or other models of brain regeneration have not been identified. In **paper III** we test the hypothesis that the newts' ability to regenerate brain tissue is connected with the ability to survive conditions of low oxygen supply, referred to as hypoxia, for extended periods of time.

A common feature among animals that can regenerate brain tissue is that they are also able to survive variation in oxygen levels. The crucian carp (*Carassius carassius*) for instance, which has been shown to regenerate whole lobes of the optic tectum, can survive for months in hypoxic water and is subjected to these conditions in its natural habitat (Kirsche and Kirsche, 1961; Nilsson and Renshaw, 2004). Zebrafish, which can also regenerate their brain, are able survive for days in a hypoxic environment (Cao et al., 2008; Kroehne et al., 2011).

Amongst reptiles, the turtle is the most studied hypoxia-tolerant animal. The Western painted turtle (*Chrysemys picta*), can survive for up to four months without oxygen during winter dormancy. There are also studies suggesting that turtles can regenerate their brain, but this has not yet been extensively studied (Kesaraju and Milton, 2009; Ultsch, 1985).

To create a hypoxic environment for the newt we made use of a system that has previously been used to study the effect of hypoxia on zebrafish (Cao et al., 2010). In this protocol, nitrogen is perfused into the aquarium in a controlled manner and the oxygen tension can be regulated with a high degree of precision.

When the oxygen tension was directly lowered to 20% of control levels, animals did not remain conscious, but if the oxygen tension was gradually lowered to 20% over 2 days, at least 66% of the animals were able to survive 5 days of hypoxia. Since not all animals survived, it is probable that this treatment caused tissue damage to organs highly dependent oxygen, such as the brain. To examine if there was an increase in cell death after the hypoxia treatment, TUNEL staining was performed on brain sections and a 6-fold increase in $TUNEL^+$ cells was observed compared to control animals, thus showing that although these animals are able to survive hypoxia, this treatment causes damage to the brain.

To test if hypoxia or reoxygenation affects proliferation of progenitor cells, immunohistochemistry was performed using antibodies against the proliferation marker PCNA and the $PCNA^+$ ventricular progenitor cells quantified. Interestingly, 5 days of hypoxia did not alter proliferation rate and neither did placing animals at 200% of oxygen tension. But when animals were reoxygenated either in normoxia or at 200% oxygen tension, this increased proliferation of the progenitor cells, suggesting that the rise in oxygen tension activates the progenitor cells.

An earlier study by our group has shown that there is an inflammatory response and activation of microglia cells, after injury to the newt midbrain (Kirkham et al., 2011). To examine if microglia react to the damage caused by hypoxia or reoxygenation, we performed double staining for PCNA and the microglial marker ionized calcium binding adaptor molecule 1 (IBA1). After reoxygenation of the newt brain, there was an increase in the number of proliferating microglia cells, showing that reoxygenation after hypoxia induces an inflammatory response.

Reoxygenation of tissues is known to increase levels of reactive oxygen species (ROS) in various organs, importantly ROS can be damaging to the tissue, but they can also functions as signalling molecules (D'Autreaux and Toledano, 2007; Vanden Hoek et al., 1998). A recent study has shown that endogenous ROS levels control proliferation of neural progenitor cells in the SVX of mice (Le Belle et al., 2011). To examine if reoxygenation results in increased ROS production, animals were injected with the ROS sensitive dye hydroethidine (HEt). After three hours of reoxygenation an increase in HEt signal was observed in both ventricular and parenchymal cells, showing that reoxygenation leads to an increased production of ROS. Since reoxygenation induces an increase in the proliferation of progenitor cells, we wanted to examine if this increase is related to higher levels of ROS. A major source for ROS is nicotinamide adenine dinucleotide phosphate-oxidase (NADPH-oxidase or NOX), which can be

inhibited by the drug apocynin (Muijsers et al., 2000). While apocynin treatment did not alter progenitor cell proliferation in control animals it induced a 46% decrease in animals subjected to reoxygenation, suggesting that the proliferative effect observed during reoxygenation is at least partially due to an increase in ROS production.

These results show that the red spotted newt is able to survive hypoxia and that this treatment leads to increased cell death. Reoxygenation of the brain induces regenerative mechanism, such as an immune response and increased proliferation of neural progenitor cells.

4.4.2 Future experiments and discussion

Future experiments in this project should firstly investigate the identity of the cells that die during hypoxia and reoxygenation in the newt brain. To examine this, one could perform immunohistochemistry for markers for cell death such as different caspases, and neuron and glial specific markers. It is also necessary to test if the increase in proliferation observed during reoxygenation leads to an increased production of neurons or other supporting cells. This could be examined by treating animals with BrdU during reoxygenation, and examine the phenotype of the $BrdU^+$ cells after a chase period.

It would be interesting to examine if ROS signalling is involved in activation of progenitor cells during regeneration of DA neurons in the newt brain. To test this I would be possible to treat 6-OHDA lesioned animals with apocynin during regeneration.

To further test the hypothesis that the ability of some vertebrates to regenerate brain tissue, is linked to their ability to survive hypoxia, experiments need to be replicated in other model organisms, for example fish.

Finally, it would be interesting to examine if sub-lethal systemic hypoxia induces damage to heart tissue. Both newts and zebrafish are able to regenerate heart and ROS signalling has previously been shown to control cardiomyocyte behaviour (Jopling et al., 2010; Witman et al., 2011). If similar mechanism of regeneration are involved in brain and heart regeneration, this would increase our understanding of vertebrate regeneration, and have implications for the development of regenerative therapies for degenerative diseases.

4.5 CONCLUDING REMARKS

In this thesis I have shown that the red spotted newt can regenerate its midbrain DA neurons following injury in a process that involves activation of quiescent RGLs. The regeneration of DA neurons is under the control of dopamine, and altering the levels of dopamine signalling in the brain effects the proliferation of the RGLs. Finally, I have shown that the red spotted newt is able to survive in hypoxia and that reoxygenation activates regenerative mechanisms in the brain.

I hope that anyone who has read this far in my thesis agrees with me when I state that the red spotted newt is an amazing model system with which to study brain regeneration. The current drawbacks of this animal model, such as lack of genomic information, could be overcomed at little cost thanks to the development of high speed, whole genome sequencing techniques. There are also advantages to this model that were not touched upon in this thesis. For example, the morphology and size of the brain is ideal for whole-mount imagining techniques (see Figure 3F, **Paper II**), which could be used to study the integration of new neurons into otherwise, already established neural networks. An effort should also be made to elucidate the mechanisms of axon guidance and synaptogenesis in an adult context, as this might reveal how we can aid regeneration of neural networks in the human brain.

Much research is now aimed at developing cell replacement therapies for injured and degenerated tissues. Results presented in this thesis could help the development of protocols for progenitor cell differentiation *in vitro,* and may have implications for the treatment of Parkinsons disease and other neurodegenerative diseases of the brain.

5 ACKNOWLEDGEMENTS

I would like to especially thank the following people:

My supervisor **András Simon**, thanks accepting me as your PhD student and for introducing me to the field of regenerative biology. It has been a true pleasure to discuss my projects and science in general. Thanks for letting me follow my own interests and for guiding me in the right directions.

Current and past members of the **Simon** lab:
Matthew Kirkham, who plastered my bleeding fingers after my first session with the cryostat and took care of me when I was a nervous newcomer to the lab. Thanks for good collaborations and always staying calm, even though I maybe didn't.

Paula Borg, thanks for being a good friend, it was sad when you left. **Laure Belnoue,** thanks for nice tea breaks and keeping me up to date on neurogenesis in the olfactory bulb and teaching me French. **Goncalo Brito,** for letting me discuss my ailments without embarrassment. **Wang Heng**, makes the best dumplings in Sweden, thanks for nice lunches and endless discussion of sport, thanks for teaching me some cloning. **Shahul Hameed,** thanks for the great food you bring to the lunchroom. **Sara Lööf**, for discussions on science and gossip. **Jamie Morrison,** for keeping me on my toes during my first seminars and giving good advice of science and life in general.

Ivana Bulatovic, Thanks for giving us a clinical approach in our lab discussions and for always having a smile to share.

Fredrik Lanner for creating a welcoming atmosphere in the lab, and good luck in your new lab!

Members of the **Frisén** lab:
Jonas Frisén, for sharing your lab space and reagents with us and for scientific input in my work. **Helena Lönnqvist,** for making sure our lab doesn't look like a pigsty and helping us in the ever so important, everyday problems. **James Chell,** for putting up with endless Auden recitations and sharing the dream to on day be able to go undercover and examine what's on the other side of the iron curtain.
To all the members of the Frisén lab, thanks for being good neighbors and thanks nice discussions in the corridor.

Members of the **Arenas** lab:
Ernest Arenas, thank you for being my co-supervisor and for inspiring meetings. **Spyridon Theofilopoulos** and **Carmen Saltó,** thanks for good collaborations and nice discussions.

Members of the **Cao** lab:
Yihai Cao, Thanks for letting me use your equipment and methods. **Lasse Jensen** thanks for teaching me how to use the hypoxia setup, and spending time with the newts, it was a great help!

Members of the **Dantuma** lab: Thanks for the barbecues and fikas, I promise I will bring some cake one day soon. Special thanks to **Florian Salomons** for making sure my confocal skills are up to date.

Thank you everyone at **CMB** for creating a nice and happy workplace. I wont forget the smiles that met me every day and made me happy. **Pauline Ajok,** for being a good friend and cheering me up everyday. **Victoria Menendez**, thanks for always smiling, and being a good running partner.

Thanks to the members of **DBRM** for the inspiring Wednesday seminars. **Urban Lendahl,** thank you for creating an excellent centre of excellence, and helping me with the decisions concerning my postdoc. **Matti Nikkola,** for always being helpful and encouraging, since my first lecture at the Uppsala University to the last panic-stricken days before my thesis defense.

Kári Ólafsson, thanks for Macdonald lunches and friendship. And thank you to my other friends in Uppsala, **Carl-Olof Joachimsson** and **Magnus Norberg.**
Maja Malmberg, thanks for nice lunch and being a good friend, I promise I will be more proactive in the future**.**

Thanks to the **Bott** family**, Oliver** and **Sigrun,** for letting me be part of your family and introducing me to the culinary delights of Germany, but more importantly the beer.

I would like to thank my **family,** who are always supportive**,**
In England: **Mummy, Barry, Emma and Jamie.**
In Sweden**: Pappa, Lisbeth, Nicholas and Åsa.**

Special thanks to **Åsa** for lending me Campbell's Biology in 1997 and always being encouraging and inspiring. I would not be a scientist if it were not for you. Thank you.

And to all my nieces, who are all lovely and beautiful:
Stella, Peggy, Vanna, Nell, and little **Amy.**

Finally **Laura,**
My North, my South, my East and West,
My working week and my Sunday rest.
Thank you.

6 REFERENCES

Able, D.J. (1999). Scramble competition selects for greater tailfin size in male red-spotted newts (Amphibia : Salamandridae). Behav Ecol Sociobiol *46*, 423-428.

Adolf, B., Chapouton, P., Lam, C.S., Topp, S., Tannhauser, B., Strahle, U., Gotz, M., and Bally-Cuif, L. (2006). Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. Dev Biol *295*, 278-293.

Agata, K., and Inoue, T. (2012). Survey of the differences between regenerative and non-regenerative animals. Development, growth & differentiation *54*, 143-152.

Agata, K., Saito, Y., and Nakajima, E. (2007). Unifying principles of regeneration I: Epimorphosis versus morphallaxis. Development, growth & differentiation *49*, 73-78.

Alabed, S., Latifeh, Y., Mohammad, H.A., and Rifai, A. (2011). Gamma-aminobutyric acid agonists for neuroleptic-induced tardive dyskinesia. Cochrane Database Syst Rev, CD000203.

Alonso, M., Lepousez, G., Wagner, S., Bardy, C., Gabellec, M.M., Torquet, N., and Lledo, P.M. (2012). Activation of adult-born neurons facilitates learning and memory. Nat Neurosci.

Altman, J. (1962). Are new neurons formed in the brains of adult mammals? Science *135*, 1127-1128.

Altman, J. (1969). Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol *137*, 433-457.

Altman, J., and Das, G.D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol *124*, 319-335.

Alvarez-Buylla, A. (1990). Mechanism of neurogenesis in adult avian brain. Experientia *46*, 948-955.

Alvarez-Buylla, A., Theelen, M., and Nottebohm, F. (1990). Proliferation "hot spots" in adult avian ventricular zone reveal radial cell division. Neuron *5*, 101-109.

Anchelin, M., Murcia, L., Alcaraz-Perez, F., Garcia-Navarro, E.M., and Cayuela, M.L. (2011). Behaviour of telomere and telomerase during aging and regeneration in zebrafish. PLoS One *6*, e16955.

Andang, M., Hjerling-Leffler, J., Moliner, A., Lundgren, T.K., Castelo-Branco, G., Nanou, E., Pozas, E., Bryja, V., Halliez, S., Nishimaru, H*., et al.* (2008). Histone H2AX-dependent GABA(A) receptor regulation of stem cell proliferation. Nature *451*, 460-464.

Anderson, H., and French, V. (1985). Cell division during intercalary regeneration in the cockroach leg. J Embryol Exp Morphol *90*, 57-78.

Andersson, E., Tryggvason, U., Deng, Q., Friling, S., Alekseenko, Z., Robert, B., Perlmann, T., and Ericson, J. (2006). Identification of intrinsic determinants of midbrain dopamine neurons. Cell *124*, 393-405.

Aponso, P.M., Faull, R.L., and Connor, B. (2008). Increased progenitor cell proliferation and astrogenesis in the partial progressive 6-hydroxydopamine model of Parkinson's disease. Neuroscience *151*, 1142-1153.

Arakawa, Y., Sendtner, M., and Thoenen, H. (1990). Survival effect of ciliary neurotrophic factor (CNTF) on chick embryonic motoneurons in culture: comparison with other neurotrophic factors and cytokines. J Neurosci *10*, 3507-3515.

Arenas, E. (2005). Engineering a dopaminergic phenotype in stem/precursor cells: role of Nurr1, glia-derived signals, and Wnts. Ann N Y Acad Sci *1049*, 51-66.

Aristotle (1943). Generation of Animals. (William Heinemann Ltd. and Harvard University Press).

Aristotle (1984). Historia Animalium (Bk. 1-3) (Harvard University Press).

Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z., and Lindvall, O. (2002). Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med *8*, 963-970.

Arvidsson, A., Kokaia, Z., and Lindvall, O. (2001). N-methyl-D-aspartate receptor-mediated increase of neurogenesis in adult rat dentate gyrus following stroke. Eur J Neurosci *14*, 10-18.

Astley, H.C., and Roberts, T.J. (2012). Evidence for a vertebrate catapult: elastic energy storage in the plantaris tendon during frog jumping. Biol Lett *8*, 386-389.

Baker, S.A., Baker, K.A., and Hagg, T. (2004). Dopaminergic nigrostriatal projections regulate neural precursor proliferation in the adult mouse subventricular zone. Eur J Neurosci *20*, 575-579.

Barbeau, A., Dallaire, L., Buu, N.T., Poirier, J., and Rucinska, E. (1985a). Comparative behavioral, biochemical and pigmentary effects of MPTP, MPP+ and paraquat in Rana pipiens. Life Sci *37*, 1529-1538.

Barbeau, A., Dallaire, L., Buu, N.T., Veilleux, F., Boyer, H., de Lanney, L.E., Irwin, I., Langston, E.B., and Langston, J.W. (1985b). New amphibian models for the study of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Life Sci *36*, 1125-1134.

Barker, N., van Es, J.H., Kuipers, J., Kujala, P., van den Born, M., Cozijnsen, M., Haegebarth, A., Korving, J., Begthel, H., Peters, P.J., *et al.* (2007). Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature *449*, 1003-1007.

Barnea, A., and Nottebohm, F. (1994). Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. Proc Natl Acad Sci U S A *91*, 11217-11221.

Beckstead, M.J., Grandy, D.K., Wickman, K., and Williams, J.T. (2004). Vesicular dopamine release elicits an inhibitory postsynaptic current in midbrain dopamine neurons. Neuron *42*, 939-946.

Bely, A.E., and Nyberg, K.G. (2010). Evolution of animal regeneration: re-emergence of a field. Trends Ecol Evol *25*, 161-170.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabe-Heider, F., Walsh, S., Zupicich, J., Alkass, K., Buchholz, B.A., Druid, H., *et al.* (2009). Evidence for cardiomyocyte renewal in humans. Science *324*, 98-102.

Bergmann, O., Liebl, J., Bernard, S., Alkass, K., Yeung, M.S., Steier, P., Kutschera, W., Johnson, L., Landen, M., Druid, H., *et al.* (2012). The age of olfactory bulb neurons in humans. Neuron *74*, 634-639.

Berner, N.J., and Puckett, R.E. (2010). Phenotypic flexibility and thermoregulatory behavior in the eastern red-spotted newt (Notophthalmus viridescens viridescens). J Exp Zool A Ecol Genet Physiol *313*, 231-239.

Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V., and Greenamyre, J.T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci *3*, 1301-1306.

Bhardwaj, R.D., Curtis, M.A., Spalding, K.L., Buchholz, B.A., Fink, D., Bjork-Eriksson, T., Nordborg, C., Gage, F.H., Druid, H., Eriksson, P.S., *et al.* (2006). Neocortical neurogenesis in humans is restricted to development. Proc Natl Acad Sci U S A *103*, 12564-12568.

Birnbaum, K.D., and Sanchez Alvarado, A. (2008). Slicing across kingdoms: regeneration in plants and animals. Cell *132*, 697-710.

Bjorklund, A., and Lindvall, O. (1975). Dopamine in dendrites of substantia nigra neurons: suggestions for a role in dendritic terminals. Brain Res *83*, 531-537.

Bjorklund, A., and Lindvall, O. (2000). Cell replacement therapies for central nervous system disorders. Nat Neurosci *3*, 537-544.

Blankenship, A.G., and Feller, M.B. (2010). Mechanisms underlying spontaneous patterned activity in developing neural circuits. Nat Rev Neurosci *11*, 18-29.

Bonaguidi, M.A., Song, J., Ming, G.L., and Song, H. (2012). A unifying hypothesis on mammalian neural stem cell properties in the adult hippocampus. Curr Opin Neurobiol.

Bonaguidi, M.A., Wheeler, M.A., Shapiro, J.S., Stadel, R.P., Sun, G.J., Ming, G.L., and Song, H. (2011). In vivo clonal analysis reveals self-renewing and multipotent adult neural stem cell characteristics. Cell *145*, 1142-1155.

Bongarzone, E.R., Howard, S.G., Schonmann, V., and Campagnoni, A.T. (1998). Identification of the dopamine D3 receptor in oligodendrocyte precursors: potential role in regulating differentiation and myelin formation. J Neurosci *18*, 5344-5353.

Bonilla, S., Hall, A.C., Pinto, L., Attardo, A., Gotz, M., Huttner, W.B., and Arenas, E. (2008). Identification of midbrain floor plate radial glia-like cells as dopaminergic progenitors. Glia *56*, 809-820.

Brickley, S.G., and Mody, I. (2012). Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. Neuron *73*, 23-34.

Brill, M.S., Ninkovic, J., Winpenny, E., Hodge, R.D., Ozen, I., Yang, R., Lepier, A., Gascon, S., Erdelyi, F., Szabo, G., *et al.* (2009). Adult generation of glutamatergic olfactory bulb interneurons. Nat Neurosci *12*, 1524-1533.

Brockes, J., and Kumar, A. (2005). Newts. Curr Biol *15*, R42-44.

Brockes, J.P. (1997). Amphibian limb regeneration: rebuilding a complex structure. Science *276*, 81-87.

Brockes, J.P., and Kumar, A. (2008). Comparative aspects of animal regeneration. Annu Rev Cell Dev Biol *24*, 525-549.

Brockes, J.P., Kumar, A., and Velloso, C.P. (2001). Regeneration as an evolutionary variable. J Anat *199*, 3-11.

Buck, L.B. (1996). Information coding in the vertebrate olfactory system. Annu Rev Neurosci *19*, 517-544.

Bystron, I., Blakemore, C., and Rakic, P. (2008). Development of the human cerebral cortex: Boulder Committee revisited. Nat Rev Neurosci *9*, 110-122.

Cabelguen, J.M., Ijspeert, A., Lamarque, S., and Ryczko, D. (2010). Axial dynamics during locomotion in vertebrates lesson from the salamander. Prog Brain Res *187*, 149-162.

Cairns, J. (1975). Mutation selection and the natural history of cancer. Nature *255*, 197-200.

Cameron, H.A., McEwen, B.S., and Gould, E. (1995). Regulation of adult neurogenesis by excitatory input and NMDA receptor activation in the dentate gyrus. J Neurosci *15*, 4687-4692.

Cameron, H.A., and McKay, R.D. (2001). Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol *435*, 406-417.

Cancedda, L., Fiumelli, H., Chen, K., and Poo, M.M. (2007). Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. J Neurosci *27*, 5224-5235.

Cao, J., Wenberg, K., and Cheng, M.F. (2002). Lesion induced new neuron incorporation in the adult hypothalamus of the avian brain. Brain Res *943*, 80-92.

Carlen, M., Meletis, K., Goritz, C., Darsalia, V., Evergren, E., Tanigaki, K., Amendola, M., Barnabe-Heider, F., Yeung, M.S., Naldini, L*., et al.* (2009). Forebrain ependymal cells are Notch-dependent and generate neuroblasts and astrocytes after stroke. Nat Neurosci *12*, 259-267.

Carleton, A., Petreanu, L.T., Lansford, R., Alvarez-Buylla, A., and Lledo, P.M. (2003). Becoming a new neuron in the adult olfactory bulb. Nat Neurosci *6*, 507-518.

Carlsson, A., Lindqvist, M., Magnusson, T., and Waldeck, B. (1958). On the presence of 3-hydroxytyramine in brain. Science *127*, 471.

Castro, D.S., Martynoga, B., Parras, C., Ramesh, V., Pacary, E., Johnston, C., Drechsel, D., Lebel-Potter, M., Garcia, L.G., Hunt, C*., et al.* (2011). A novel function of the proneural factor Ascl1 in progenitor proliferation identified by genome-wide characterization of its targets. Genes Dev *25*, 930-945.

Chan, P., Di Monte, D.A., Langston, J.W., and Janson, A.M. (1997). (+)MK-801 does not prevent MPTP-induced loss of nigral neurons in mice. J Pharmacol Exp Ther *280*, 439-446.

Chapouton, P., Jagasia, R., and Bally-Cuif, L. (2007). Adult neurogenesis in non-mammalian vertebrates. Bioessays *29*, 745-757.

Charge, S.B.P., and Rudnicki, M.A. (2004). Cellular and molecular regulation of muscle regeneration. Physiol Rev *84*, 209-238.

Chen, Z.L., Yu, W.M., and Strickland, S. (2007). Peripheral regeneration. Annu Rev Neurosci *30*, 209-233.

Chera, S., Ghila, L., Dobretz, K., Wenger, Y., Bauer, C., Buzgariu, W., Martinou, J.C., and Galliot, B. (2009). Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. Dev Cell *17*, 279-289.

Chetverukhin, V.K., and Polenov, A.L. (1993). Ultrastructural radioautographic analysis of neurogenesis in the hypothalamus of the adult frog, Rana temporaria, with special reference to physiological regeneration of the preoptic nucleus. I. Ventricular zone cell proliferation. Cell Tissue Res *271*, 341-350.

Chevallier, S., Jan Ijspeert, A., Ryczko, D., Nagy, F., and Cabelguen, J.M. (2008). Organisation of the spinal central pattern generators for locomotion in the salamander: biology and modelling. Brain Res Rev *57*, 147-161.

Collins, C.A., Olsen, I., Zammit, P.S., Heslop, L., Petrie, A., Partridge, T.A., and Morgan, J.E. (2005). Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell *122*, 289-301.

Colucci-D'Amato, L., Bonavita, V., and di Porzio, U. (2006). The end of the central dogma of neurobiology: stem cells and neurogenesis in adult CNS. Neurol Sci *27*, 266-270.

Coronas, V., Bantubungi, K., Fombonne, J., Krantic, S., Schiffmann, S.N., and Roger, M. (2004). Dopamine D3 receptor stimulation promotes the proliferation of cells derived from the post-natal subventricular zone. J Neurochem *91*, 1292-1301.

Curtis, M.A., Kam, M., Nannmark, U., Anderson, M.F., Axell, M.Z., Wikkelso, C., Holtas, S., van Roon-Mom, W.M., Bjork-Eriksson, T., Nordborg, C*., et al.* (2007). Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science *315*, 1243-1249.

Dahlgren, E.W., Vetenskapsakademien, K.S., and . (1915). Kungl. Svenska vetenskapsakademien, Personförteckningar, 1739-1915 (Almqvist & Wiksells boktr.).

Davis, B.M., Ayers, J.L., Koran, L., Carlson, J., Anderson, M.C., and Simpson, S.B., Jr. (1990). Time course of salamander spinal cord regeneration and recovery of swimming: HRP retrograde pathway tracing and kinematic analysis. Exp Neurol *108*, 198-213.

Davis, G.K. (2012). Cyclical Parthenogenesis and Viviparity in Aphids as Evolutionary Novelties. J Exp Zool B Mol Dev Evol.

Dawirs, R.R., Hildebrandt, K., and Teuchert-Noodt, G. (1998). Adult treatment with haloperidol increases dentate granule cell proliferation in the gerbil hippocampus. J Neural Transm *105*, 317-327.

Dawson, T.M., and Dawson, V.L. (2003). Molecular pathways of neurodegeneration in Parkinson's disease. Science *302*, 819-822.

Dayer, A.G., Cleaver, K.M., Abouantoun, T., and Cameron, H.A. (2005). New GABAergic interneurons in the adult neocortex and striatum are generated from different precursors. J Cell Biol *168*, 415-427.

De Robertis, E.M. (2006). Spemann's organizer and self-regulation in amphibian embryos. Nat Rev Mol Cell Biol *7*, 296-302.

Desplanches, D., Mayet, M.H., Ilyina-Kakueva, E.I., Sempore, B., and Flandrois, R. (1990). Skeletal muscle adaptation in rats flown on Cosmos 1667. J Appl Physiol *68*, 48-52.

Deumens, R., Blokland, A., and Prickaerts, J. (2002). Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. Exp Neurol *175*, 303-317.

Dexter, D.T., Carter, C.J., Wells, F.R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., and Marsden, C.D. (1989). Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. J Neurochem *52*, 381-389.

Diaz, J., Ridray, S., Mignon, V., Griffon, N., Schwartz, J.C., and Sokoloff, P. (1997). Selective expression of dopamine D3 receptor mRNA in proliferative zones during embryonic development of the rat brain. J Neurosci *17*, 4282-4292.

Dinsmore, C.E.e. (1991). A history of regeneration research. Milestones in the Evolution of a Science (Cambridge University Press).

Doetsch, F., Caille, I., Lim, D.A., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (1999a). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell *97*, 703-716.

Doetsch, F., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (1999b). Regeneration of a germinal layer in the adult mammalian brain. Proc Natl Acad Sci U S A *96*, 11619-11624.

Duggal, N., Schmidt-Kastner, R., and Hakim, A.M. (1997). Nestin expression in reactive astrocytes following focal cerebral ischemia in rats. Brain Res *768*, 1-9.

Echeverri, K., Clarke, J.D., and Tanaka, E.M. (2001). In vivo imaging indicates muscle fiber dedifferentiation is a major contributor to the regenerating tail blastema. Dev Biol *236*, 151-164.

Echeverri, K., and Tanaka, E.M. (2005). Proximodistal patterning during limb regeneration. Dev Biol *279*, 391-401.

Eguchi, G. (1988). Cellular and molecular background of wolffian lens regeneration. Cell Differ Dev *25 Suppl*, 147-158.

Eguchi, G., Eguchi, Y., Nakamura, K., Yadav, M.C., Millan, J.L., and Tsonis, P.A. (2011). Regenerative capacity in newts is not altered by repeated regeneration and ageing. Nat Commun *2*, 384.

Ehninger, D., and Kempermann, G. (2003). Regional effects of wheel running and environmental enrichment on cell genesis and microglia proliferation in the adult murine neocortex. Cereb Cortex *13*, 845-851.

Elger, M., Hentschel, H., Litteral, J., Wellner, M., Kirsch, T., Luft, F.C., and Haller, H. (2003). Nephrogenesis is induced by partial nephrectomy in the elasmobranch Leucoraja erinacea. J Am Soc Nephrol *14*, 1506-1518.

Elsworth, J.D., and Roth, R.H. (1997). Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease. Exp Neurol *144*, 4-9.

Emilien, G., Maloteaux, J.M., Geurts, M., Hoogenberg, K., and Cragg, S. (1999). Dopamine receptors--physiological understanding to therapeutic intervention potential. Pharmacol Ther *84*, 133-156.

Emsley, J.G., and Hagg, T. (2003). Endogenous and exogenous ciliary neurotrophic factor enhances forebrain neurogenesis in adult mice. Exp Neurol *183*, 298-310.

Endepols, H., Schul, J., Gerhardt, H.C., and Walkowiak, W. (2004). 6-hydroxydopamine lesions in anuran amphibians: a new model system for Parkinson's disease? J Neurobiol *60*, 395-410.

Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., and Gage, F.H. (1998). Neurogenesis in the adult human hippocampus. Nat Med *4*, 1313-1317.

Falconer, E., Chavez, E.A., Henderson, A., Poon, S.S., McKinney, S., Brown, L., Huntsman, D.G., and Lansdorp, P.M. (2010). Identification of sister chromatids by DNA template strand sequences. Nature *463*, 93-97.

Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A., and Sklar, P. (2005). Molecular genetics of attention-deficit/hyperactivity disorder. Biol Psychiatry *57*, 1313-1323.

Ferre, S., Baler, R., Bouvier, M., Caron, M.G., Devi, L.A., Durroux, T., Fuxe, K., George, S.R., Javitch, J.A., Lohse, M.J*., et al.* (2009). Building a new conceptual framework for receptor heteromers. Nat Chem Biol *5*, 131-134.

Ferretti, P. (2011). Is there a relationship between adult neurogenesis and neuron generation following injury across evolution? Eur J Neurosci *34*, 951-962.

Ferretti, P., and Brockes, J.P. (1988). Culture of newt cells from different tissues and their expression of a regeneration-associated antigen. J Exp Zool *247*, 77-91.

Finch, C.E., and Austad, S.N. (2011). Blind cave salamanders age very slowly: a new member of Methuselah's Bestiary. Bioessays *33*, 27-29.

Flemming, W. (1882). Zellsubstanz, Kern und Zelltheilung (Vogel).

Font, E., Desfilis, E., Perez-Canellas, M.M., and Garcia-Verdugo, J.M. (2001). Neurogenesis and neuronal regeneration in the adult reptilian brain. Brain Behav Evol *58*, 276-295.

Font, E., Garcia-Verdugo, J.M., Alcantara, S., and Lopez-Garcia, C. (1991). Neuron regeneration reverses 3-acetylpyridine-induced cell loss in the cerebral cortex of adult lizards. Brain Res *551*, 230-235.

Freundlieb, N., Francois, C., Tande, D., Oertel, W.H., Hirsch, E.C., and Hoglinger, G.U. (2006). Dopaminergic substantia nigra neurons project topographically organized to the subventricular zone and stimulate precursor cell proliferation in aged primates. J Neurosci *26*, 2321-2325.

Frielingsdorf, H., Schwarz, K., Brundin, P., and Mohapel, P. (2004). No evidence for new dopaminergic neurons in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A *101*, 10177-10182.

Frolich, L.M., and Biewener, A.A. (1992). Kinematic and Electromyographic Analysis of the Functional-Role of the Body Axis during Terrestrial and Aquatic Locomotion in the Salamander Ambystoma-Tigrinum. J Exp Biol *162*, 107-130.

Fujisawa, T. (2003). Hydra regeneration and epitheliopeptides. Dev Dyn *226*, 182-189.

Fukuda, S., Kato, F., Tozuka, Y., Yamaguchi, M., Miyamoto, Y., and Hisatsune, T. (2003). Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. J Neurosci *23*, 9357-9366.

Gage, F.H. (2000). Mammalian neural stem cells. Science *287*, 1433-1438.

Gao, K.Q., and Shubin, N.H. (2001). Late Jurassic salamanders from northern China. Nature *410*, 574-577.

Garcia-Verdugo, J.M., Ferron, S., Flames, N., Collado, L., Desfilis, E., and Font, E. (2002). The proliferative ventricular zone in adult vertebrates: a comparative study using reptiles, birds, and mammals. Brain Res Bull *57*, 765-775.

Garcia-Verdugo, J.M., Llahi, S., Ferrer, I., and Lopez-Garcia, C. (1989). Postnatal neurogenesis in the olfactory bulbs of a lizard. A tritiated thymidine autoradiographic study. Neurosci Lett *98*, 247-252.

Garza-Garcia, A.A., Driscoll, P.C., and Brockes, J.P. (2010). Evidence for the local evolution of mechanisms underlying limb regeneration in salamanders. Integr Comp Biol *50*, 528-535.

Gazenko, O.G., and Ilyin, E.A. (1986). Investigations onboard the biosatellite Cosmos-1667. Adv Space Res *6*, 101-106.

Geffen, L.B., Jessell, T.M., Cuello, A.C., and Iversen, L.L. (1976). Release of dopamine from dendrites in rat substantia nigra. Nature *260*, 258-260.

Giros, B., Jaber, M., Jones, S.R., Wightman, R.M., and Caron, M.G. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature *379*, 606-612.

Godard, R. (1991). Long-Term-Memory of Individual Neighbors in a Migratory Songbird. Nature *350*, 228-229.

Goldman, S.A., and Nottebohm, F. (1983). Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. Proc Natl Acad Sci U S A *80*, 2390-2394.

Goss, R.J. (1992). The evolution of regeneration: adaptive or inherent? J Theor Biol *159*, 241-260.

Goss, R.J., and Stagg, M.W. (1958). REGENERATION IN LOWER JAWS OF NEWTS AFTER EXCISION OF THE INTERMANDIBULAR REGIONS. J Exp Zool *137*, 1-11.

Gould, E. (2007). How widespread is adult neurogenesis in mammals? Nat Rev Neurosci *8*, 481-488.

Gould, E., Reeves, A.J., Graziano, M.S., and Gross, C.G. (1999). Neurogenesis in the neocortex of adult primates. Science *286*, 548-552.

Grandel, H., Kaslin, J., Ganz, J., Wenzel, I., and Brand, M. (2006). Neural stem cells and neurogenesis in the adult zebrafish brain: origin, proliferation dynamics, migration and cell fate. Dev Biol *295*, 263-277.

Gratzner, H.G. (1982). Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: A new reagent for detection of DNA replication. Science *218*, 474-475.

Grillner, S., and Jessell, T.M. (2009). Measured motion: searching for simplicity in spinal locomotor networks. Curr Opin Neurobiol *19*, 572-586.

Gross, C.G. (2009). Three before their time: neuroscientists whose ideas were ignored by their contemporaries. Exp Brain Res *192*, 321-334.

Guzman, J.N., Sanchez-Padilla, J., Chan, C.S., and Surmeier, D.J. (2009). Robust pacemaking in substantia nigra dopaminergic neurons. J Neurosci *29*, 11011-11019.

Guzman, J.N., Sanchez-Padilla, J., Wokosin, D., Kondapalli, J., Ilijic, E., Schumacker, P.T., and Surmeier, D.J. (2010). Oxidant stress evoked by pacemaking in dopaminergic neurons is attenuated by DJ-1. Nature *468*, 696-700.

Hahnloser, R.H., Kozhevnikov, A.A., and Fee, M.S. (2002). An ultra-sparse code underlies the generation of neural sequences in a songbird. Nature *419*, 65-70.

Halim, N.D., Weickert, C.S., McClintock, B.W., Weinberger, D.R., and Lipska, B.K. (2004). Effects of chronic haloperidol and clozapine treatment on neurogenesis in the adult rat hippocampus. Neuropsychopharmacology *29*, 1063-1069.

Hayflick, L., and Moorhead, P.S. (1961). The serial cultivation of human diploid cell strains. Exp Cell Res *25*, 585-621.

Hillman, S.S., Withers, P.C., Drewes, C.D., and Hillyard, S.D. (2009). Ecological and environmental physiology of amphibians Vol 1 (Oxford ; New York : Oxford University Press, 2009).

Hiyama, E., and Hiyama, K. (2007). Telomere and telomerase in stem cells. Br J Cancer *96*, 1020-1024.

Hoglinger, G.U., Rizk, P., Muriel, M.P., Duyckaerts, C., Oertel, W.H., Caille, I., and Hirsch, E.C. (2004). Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat Neurosci *7*, 726-735.

Holstein, T.W., Hobmayer, E., and David, C.N. (1991). Pattern of epithelial cell cycling in hydra. Dev Biol *148*, 602-611.

Hsu, Y.C., and Fuchs, E. (2012). A family business: stem cell progeny join the niche to regulate homeostasis. Nat Rev Mol Cell Biol *13*, 103-114.

Hughes, R.N. (1989). A Functional Biology of Clonal Animals (Chapman and Hall).

Hughes, W.L., Bond, V.P., Brecher, G., Cronkite, E.P., Painter, R.B., Quastler, H., and Sherman, F.G. (1958). Cellular Proliferation in the Mouse as Revealed by Autoradiography with Tritiated Thymidine. Proc Natl Acad Sci U S A *44*, 476-483.

Hurlbert, S. (1969). The Breeding Migrations and Interhabitat Wandering of the Vermilion-Spotted Newt Notophthalmus viridescens (Rafinesque) Ecological Monographs
39, 465-488.

Huxley, T.H., and Huxley, L. (1900). Life and letters of Thomas Henry Huxley (D. Appleton and Company).

Ibi, D., Takuma, K., Koike, H., Mizoguchi, H., Tsuritani, K., Kuwahara, Y., Kamei, H., Nagai, T., Yoneda, Y., Nabeshima, T.*, et al.* (2008). Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice. J Neurochem *105*, 921-932.

Ijspeert, A.J., Crespi, A., Ryczko, D., and Cabelguen, J.M. (2007). From swimming to walking with a salamander robot driven by a spinal cord model. Science *315*, 1416-1420.

Illis, L.S. (2012). Central nervous system regeneration does not occur. Spinal Cord *50*, 259-263.

Imayoshi, I., Sakamoto, M., Ohtsuka, T., Takao, K., Miyakawa, T., Yamaguchi, M., Mori, K., Ikeda, T., Itohara, S., and Kageyama, R. (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. Nat Neurosci *11*, 1153-1161.

Iten, L.E., and Bryant, S.V. (1976). STAGES OF TAIL REGENERATION IN ADULT NEWT, NOTOPHTHALMUS-VIRIDESCENS. J Exp Zool *196*, 283-292.

Jopling, C., Boue, S., and Izpisua Belmonte, J.C. (2011). Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. Nat Rev Mol Cell Biol *12*, 79-89.

Jopling, C., Sleep, E., Raya, M., Marti, M., Raya, A., and Izpisua Belmonte, J.C. (2010). Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. Nature *464*, 606-609.

Kara, T.C. (1994). Ageing in amphibians. Gerontology *40*, 161-173.

Karpowicz, P., Morshead, C., Kam, A., Jervis, E., Ramunas, J., Cheng, V., and van der Kooy, D. (2005). Support for the immortal strand hypothesis: neural stem cells partition DNA asymmetrically in vitro. J Cell Biol *170*, 721-732.

Kaslin, J., Ganz, J., and Brand, M. (2008). Proliferation, neurogenesis and regeneration in the non-mammalian vertebrate brain. Philos Trans R Soc Lond B Biol Sci *363*, 101-122.

Kelly, P.H., Seviour, P.W., and Iversen, S.D. (1975). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res *94*, 507-522.

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., and Gage, F.H. (2003). Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development *130*, 391-399.

Kierdorf, U., and Kierdorf, H. (2011). Deer antlers - a model of mammalian appendage regeneration: an extensive review. Gerontology *57*, 53-65.

Kierdorf, U., Kierdorf, H., Schultz, M., and Rolf, H.J. (2004). Histological structure of antlers in castrated male fallow deer (Dama dama). Anat Rec A Discov Mol Cell Evol Biol *281*, 1352-1362.

Kim, S.Y., Choi, K.C., Chang, M.S., Kim, M.H., Na, Y.S., Lee, J.E., Jin, B.K., Lee, B.H., and Baik, J.H. (2006). The dopamine D2 receptor regulates the development of dopaminergic neurons via extracellular signal-regulated kinase and Nurr1 activation. J Neurosci *26*, 4567-4576.

Kippin, T.E., Kapur, S., and van der Kooy, D. (2005). Dopamine specifically inhibits forebrain neural stem cell proliferation, suggesting a novel effect of antipsychotic drugs. J Neurosci *25*, 5815-5823.

Kirn, J., O'Loughlin, B., Kasparian, S., and Nottebohm, F. (1994). Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song. Proc Natl Acad Sci U S A *91*, 7844-7848.

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., Yokochi, M., Mizuno, Y., and Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature *392*, 605-608.

Kittappa, R., Chang, W.W., Awatramani, R.B., and McKay, R.D. (2007). The foxa2 gene controls the birth and spontaneous degeneration of dopamine neurons in old age. PLoS Biol *5*, e325.

Knable, M.B., and Weinberger, D.R. (1997). Dopamine, the prefrontal cortex and schizophrenia. J Psychopharmacol *11*, 123-131.

Kokoeva, M.V., Yin, H., and Flier, J.S. (2005). Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. Science *310*, 679-683.

Koob, G.F., and Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology *24*, 97-129.

Kornack, D.R., and Rakic, P. (2001). Cell proliferation without neurogenesis in adult primate neocortex. Science *294*, 2127-2130.

Kosaka, K., Oyanagi, S., Matsushita, M., and Hori, A. (1976). Presenile dementia with Alzheimer-, Pick- and Lewy-body changes. Acta Neuropathol *36*, 221-233.

Kragl, M., Knapp, D., Nacu, E., Khattak, S., Maden, M., Epperlein, H.H., and Tanaka, E.M. (2009). Cells keep a memory of their tissue origin during axolotl limb regeneration. Nature *460*, 60-65.

Kriegstein, A., and Alvarez-Buylla, A. (2009). The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci *32*, 149-184.

Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J., and Brand, M. (2011). Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. Development *138*, 4831-4841.

Kuhn, H.G., Dickinson-Anson, H., and Gage, F.H. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci *16*, 2027-2033.

Kuhn, H.G., Winkler, J., Kempermann, G., Thal, L.J., and Gage, F.H. (1997). Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. J Neurosci *17*, 5820-5829.

Kumar, A., Velloso, C.P., Imokawa, Y., and Brockes, J.P. (2000). Plasticity of retrovirus-labelled myotubes in the newt limb regeneration blastema. Dev Biol *218*, 125-136.

Kurabuchi, S. (1990). Loss of limb regenerative capacity during postmetamorphic growth in the tree frog, Hyla arborea japonica. Growth Dev Aging *54*, 17-22.

L'Episcopo, F., Tirolo, C., Testa, N., Caniglia, S., Morale, M.C., Deleidi, M., Serapide, M.F., Pluchino, S., and Marchetti, B. (2012). Plasticity of subventricular zone neuroprogenitors in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease involves cross talk between inflammatory and Wnt/beta-catenin signaling pathways: functional consequences for neuroprotection and repair. J Neurosci *32*, 2062-2085.

Lampert, K.P. (2008). Facultative parthenogenesis in vertebrates: reproductive error or chance? Sex Dev *2*, 290-301.

Langston, J.W., Ballard, P., Tetrud, J.W., and Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science *219*, 979-980.

Laudet, V. (2011). The origins and evolution of vertebrate metamorphosis. Curr Biol *21*, R726-737.

Lee, D.A., Bedont, J.L., Pak, T., Wang, H., Song, J., Miranda-Angulo, A., Takiar, V., Charubhumi, V., Balordi, F., Takebayashi, H.*, et al.* (2012). Tanycytes of the hypothalamic median eminence form a diet-responsive neurogenic niche. Nat Neurosci *15*, 700-702.

Lendahl, U., Zimmerman, L.B., and McKay, R.D. (1990). CNS stem cells express a new class of intermediate filament protein. Cell *60*, 585-595.

Lewis, P.F., and Emerman, M. (1994). Passage through mitosis is required for oncoretroviruses but not for the human immunodeficiency virus. J Virol *68*, 510-516.

Li, J.Y., Englund, E., Holton, J.L., Soulet, D., Hagell, P., Lees, A.J., Lashley, T., Quinn, N.P., Rehncrona, S., Bjorklund, A.*, et al.* (2008). Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med *14*, 501-503.

Lie, D.C., Dziewczapolski, G., Willhoite, A.R., Kaspar, B.K., Shults, C.W., and Gage, F.H. (2002). The adult substantia nigra contains progenitor cells with neurogenic potential. J Neurosci *22*, 6639-6649.

Lindvall, O., Brundin, P., Widner, H., Rehncrona, S., Gustavii, B., Frackowiak, R., Leenders, K.L., Sawle, G., Rothwell, J.C., Marsden, C.D.*, et al.* (1990). Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. Science *247*, 574-577.

Liu, X., Wang, Q., Haydar, T.F., and Bordey, A. (2005). Nonsynaptic GABA signaling in postnatal subventricular zone controls proliferation of GFAP-expressing progenitors. Nat Neurosci *8*, 1179-1187.

Lopez-Garcia, C., Molowny, A., Garcia-Verdugo, J.M., and Ferrer, I. (1988). Delayed postnatal neurogenesis in the cerebral cortex of lizards. Brain Res *471*, 167-174.

Lugert, S., Basak, O., Knuckles, P., Haussler, U., Fabel, K., Gotz, M., Haas, C.A., Kempermann, G., Taylor, V., and Giachino, C. (2010). Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. Cell Stem Cell *6*, 445-456.

Luskin, M.B. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. Neuron *11*, 173-189.

Lutz, G.J., and Rome, L.C. (1994). Built for jumping: the design of the frog muscular system. Science *263*, 370-372.

Mackay-Sim, A. (2010). Stem cells and their niche in the adult olfactory mucosa. Arch Ital Biol *148*, 47-58.

Macklis, J.D. (2012). Human adult olfactory bulb neurogenesis? Novelty is the best policy. Neuron *74*, 595-596.

Magavi, S.S., Leavitt, B.R., and Macklis, J.D. (2000). Induction of neurogenesis in the neocortex of adult mice. Nature *405*, 951-955.

Margolese, H.C., Chouinard, G., Kolivakis, T.T., Beauclair, L., and Miller, R. (2005). Tardive dyskinesia in the era of typical and atypical antipsychotics. Part 1: pathophysiology and mechanisms of induction. Can J Psychiatry *50*, 541-547.

Marion, Z.H., and Hay, M.E. (2011). Chemical defense of the eastern newt (Notophthalmus viridescens): variation in efficiency against different consumers and in different habitats. PLoS One *6*, e27581.

Markakis, E.A., and Gage, F.H. (1999). Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. J Comp Neurol *406*, 449-460.

Marsden, C.D., and Parkes, J.D. (1977). Success and problems of long-term levodopa therapy in Parkinson's disease. Lancet *1*, 345-349.

Marthy, H.J. (2003). Developmental Biology Research in Space (Elsevier).

Matsuoka, N., Nozaki, K., Takagi, Y., Nishimura, M., Hayashi, J., Miyatake, S., and Hashimoto, N. (2003). Adenovirus-mediated gene transfer of fibroblast growth factor-2 increases BrdU-positive cells after forebrain ischemia in gerbils. Stroke *34*, 1519-1525.

Melendez-Ferro, M., Perez-Costas, E., Rodriguez-Munoz, R., Gomez-Lopez, M.P., Anadon, R., and Rodicio, M.C. (2001). GABA immunoreactivity in the olfactory bulbs of the adult sea lamprey Petromyzon marinus L. Brain Res *893*, 253-260.

Merkle, F.T., Tramontin, A.D., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (2004). Radial glia give rise to adult neural stem cells in the subventricular zone. Proc Natl Acad Sci U S A *101*, 17528-17532.

Migaud, M., Batailler, M., Segura, S., Duittoz, A., Franceschini, I., and Pillon, D. (2010). Emerging new sites for adult neurogenesis in the mammalian brain: a comparative study between the hypothalamus and the classical neurogenic zones. Eur J Neurosci *32*, 2042-2052.

Miller, O.L., Jr., and Beatty, B.R. (1969). Visualization of nucleolar genes. Science *164*, 955-957.

Minelli, G., Franceschini, V., Del Grande, P., and Ciani, F. (1987). Newly-formed neurons in the regenerating optic tectum of Triturus cristatus carnifex. Basic Appl Histochem *31*, 43-52.

Ming, G.L., and Song, H. (2005). Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci *28*, 223-250.

Ming, G.L., and Song, H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron *70*, 687-702.

Missale, C., Fiorentini, C., Collo, G., and Spano, P. (2010). The neurobiology of dopamine receptors: evolution from the dual concept to heterodimer complexes. J Recept Signal Transduct Res *30*, 347-354.

Missale, C., Nash, S.R., Robinson, S.W., Jaber, M., and Caron, M.G. (1998). Dopamine receptors: from structure to function. Physiol Rev *78*, 189-225.

Mongiat, L.A., and Schinder, A.F. (2011). Adult neurogenesis and the plasticity of the dentate gyrus network. Eur J Neurosci *33*, 1055-1061.

Morgan, G.T. (2002). Lampbrush chromosomes and associated bodies: new insights into principles of nuclear structure and function. Chromosome Res *10*, 177-200.

Morgan, T.H. (1898). Experimental studies of the regeneration of Planaria maculata. Arch Entwm Org *7*, 365-397.

Morgan, T.H. (1901a). Regeneration, Vol VII (New york, London The Macmillan Company).

Morgan, T.H. (1901b). Regeneration and Liability to Injury. Science *14*, 235-248.

Mori, T., Buffo, A., and Gotz, M. (2005). The novel roles of glial cells revisited: the contribution of radial glia and astrocytes to neurogenesis. Curr Top Dev Biol *69*, 67-99.

Morrison, J.I., Loof, S., He, P., and Simon, A. (2006). Salamander limb regeneration involves the activation of a multipotent skeletal muscle satellite cell population. J Cell Biol *172*, 433-440.

Morrison, S.J., and Spradling, A.C. (2008). Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell *132*, 598-611.

Morshead, C.M., Reynolds, B.A., Craig, C.G., McBurney, M.W., Staines, W.A., Morassutti, D., Weiss, S., and van der Kooy, D. (1994). Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron *13*, 1071-1082.

Muller, H.W., and Stoll, G. (1998). Nerve injury and regeneration: basic insights and therapeutic interventions. Curr Opin Neurol *11*, 557-562.

Noctor, S.C., Flint, A.C., Weissman, T.A., Dammerman, R.S., and Kriegstein, A.R. (2001). Neurons derived from radial glial cells establish radial units in neocortex. Nature *409*, 714-720.

Northcutt, R.G. (1981). Evolution of the telencephalon in nonmammals. Annu Rev Neurosci *4*, 301-350.

Northcutt, R.G. (1995). The forebrain of gnathostomes: in search of a morphotype. Brain Behav Evol *46*, 275-318.

Nottebohm, F. (1985). Neuronal replacement in adulthood. Ann N Y Acad Sci *457*, 143-161.

O'Keeffe, G.C., Tyers, P., Aarsland, D., Dalley, J.W., Barker, R.A., and Caldwell, M.A. (2009). Dopamine-induced proliferation of adult neural precursor cells in the mammalian subventricular zone is mediated through EGF. Proc Natl Acad Sci U S A *106*, 8754-8759.

O'Steen, W.K. (1958). Regeneration of the intestine in adult urodeles. Journal of Morphology *103*, 435-477.

Odelberg, S.J. (2004). Unraveling the molecular basis for regenerative cellular plasticity. PLoS Biol *2*, E232.

Ohtani, N., Goto, T., Waeber, C., and Bhide, P.G. (2003). Dopamine modulates cell cycle in the lateral ganglionic eminence. J Neurosci *23*, 2840-2850.

Okamoto, M., Ohsawa, H., Hayashi, T., Owaribe, K., and Tsonis, P.A. (2007). Regeneration of retinotectal projections after optic tectum removal in adult newts. Mol Vis *13*, 2112-2118.

Olanow, C.W., Goetz, C.G., Kordower, J.H., Stoessl, A.J., Sossi, V., Brin, M.F., Shannon, K.M., Nauert, G.M., Perl, D.P., Godbold, J.*, et al.* (2003). A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease. Ann Neurol *54*, 403-414.

Olanow, C.W., Kordower, J.H., Lang, A.E., and Obeso, J.A. (2009a). Dopaminergic transplantation for Parkinson's disease: current status and future prospects. Ann Neurol *66*, 591-596.

Olanow, C.W., Stern, M.B., and Sethi, K. (2009b). The scientific and clinical basis for the treatment of Parkinson disease (2009). Neurology *72*, S1-136.

Ono, Y., Nakatani, T., Sakamoto, Y., Mizuhara, E., Minaki, Y., Kumai, M., Hamaguchi, A., Nishimura, M., Inoue, Y., Hayashi, H.*, et al.* (2007). Differences in neurogenic potential in floor plate cells along an anteroposterior location: midbrain dopaminergic neurons originate from mesencephalic floor plate cells. Development *134*, 3213-3225.

Oxford English Dictionary, O. "hydra, n." (Oxford University Press).

Pack, G.T., Islami, A.H., Hubbard, J.C., and Brasfield, R.D. (1962). Regeneration of human liver after major hepatectomy. Surgery *52*, 617-623.

Parent, J.M. (2003). Injury-induced neurogenesis in the adult mammalian brain. Neuroscientist *9*, 261-272.

Parent, J.M., Yu, T.W., Leibowitz, R.T., Geschwind, D.H., Sloviter, R.S., and Lowenstein, D.H. (1997). Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci *17*, 3727-3738.

Parish, C.L., Beljajeva, A., Arenas, E., and Simon, A. (2007). Midbrain dopaminergic neurogenesis and behavioural recovery in a salamander lesion-induced regeneration model. Development *134*, 2881-2887.

Parish, C.L., Finkelstein, D.I., Drago, J., Borrelli, E., and Horne, M.K. (2001). The role of dopamine receptors in regulating the size of axonal arbors. J Neurosci *21*, 5147-5157.

Park, H.D., Ortmeyer, A.B., and Blankenbaker, D.P. (1970). Cell division during regeneration in Hydra. Nature *227*, 617-619.

Parkinson, J. (2002). An essay on the shaking palsy (Reprinted). J Neuropsychiatr Clin Neurosci *14*, 223-236.

Patapoutian, A., Wold, B.J., and Wagner, R.A. (1995). Evidence for developmentally programmed transdifferentiation in mouse esophageal muscle. Science *270*, 1818-1821.

Perez-Canellas, M.M., Font, E., and Garcia-Verdugo, J.M. (1997). Postnatal neurogenesis in the telencephalon of turtles: evidence for nonradial migration of new neurons from distant proliferative ventricular zones to the olfactory bulbs. Brain Res Dev Brain Res *101*, 125-137.

Petreanu, L., and Alvarez-Buylla, A. (2002). Maturation and death of adult-born olfactory bulb granule neurons: role of olfaction. J Neurosci *22*, 6106-6113.

Pfennig, D.W., and Collins, J.P. (1993). Kinship affects morphogenesis in cannibalistic salamanders. Nature *362*, 836-838.

Platel, J.C., Stamboulian, S., Nguyen, I., and Bordey, A. (2010). Neurotransmitter signaling in postnatal neurogenesis: The first leg. Brain Res Rev *63*, 60-71.

Polenov, A.L., and Chetverukhin, V.K. (1993). Ultrastructural radioautographic analysis of neurogenesis in the hypothalamus of the adult frog, Rana temporaria, with special reference to physiological regeneration of the preoptic nucleus. II. Types of neuronal cells produced. Cell Tissue Res *271*, 351-362.

Poss, K.D. (2010). Advances in understanding tissue regenerative capacity and mechanisms in animals. Nat Rev Genet *11*, 710-722.

Poss, K.D., Wilson, L.G., and Keating, M.T. (2002). Heart regeneration in zebrafish. Science *298*, 2188-2190.

Potten, C.S., and Loeffler, M. (1990). Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development *110*, 1001-1020.

Powell, A.J., Gates, P.B., Wylie, D., Velloso, C.P., Brockes, J.P., and Jat, P.S. (1998). Immortalization of rat embryo fibroblasts by a 3'-untranslated region. Exp Cell Res *240*, 252-262.

Price, J., Turner, D., and Cepko, C. (1987). Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. Proc Natl Acad Sci U S A *84*, 156-160.

Quyn, A.J., Appleton, P.L., Carey, F.A., Steele, R.J., Barker, N., Clevers, H., Ridgway, R.A., Sansom, O.J., and Nathke, I.S. (2010). Spindle orientation bias in gut epithelial stem cell compartments is lost in precancerous tissue. Cell Stem Cell *6*, 175-181.

Rakic, P. (1971). Guidance of neurons migrating to the fetal monkey neocortex. Brain Res *33*, 471-476.

Rakic, P. (1985). Limits of neurogenesis in primates. Science *227*, 1054-1056.

Rando, T.A., and Chang, H.Y. (2012). Aging, rejuvenation, and epigenetic reprogramming: resetting the aging clock. Cell *148*, 46-57.

Ratcliff, M.J. (2005). Experimentation, communication and patronage: a perspective on Rene-Antoine Ferchault de Reaumur (1683-1757). Biol Cell *97*, 231-233.

Reddien, P.W., and Sanchez Alvarado, A. (2004). Fundamentals of planarian regeneration. Annu Rev Cell Dev Biol *20*, 725-757.

Reimschuessel, R., Bennett, R.O., May, E.B., and Lipsky, M.M. (1990). RENAL TUBULAR CELL REGENERATION, CELL-PROLIFERATION AND CHRONIC NEPHROTOXICITY IN THE GOLDFISH CARASSIUS-AURATUS FOLLOWING EXPOSURE TO A SINGLE SUBLETHAL DOSE OF HEXACHLOROBUTADIENE. Dis Aquat Org *8*, 211-224.

Reynolds, B.A., and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science *255*, 1707-1710.

Reynolds, B.A., and Weiss, S. (1996). Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. Dev Biol *175*, 1-13.

Richter, W., and Kranz, D. (1981). [Autoradiographic investigations on postnatal proliferative activity of the telencephalic and diencephalic matrix-zones in the axolotl (Ambystoma mexicanum), with special references to the olfactory organ (author's transl)]. Z Mikrosk Anat Forsch *95*, 883-904.

Rochefort, C., Gheusi, G., Vincent, J.D., and Lledo, P.M. (2002). Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. J Neurosci *22*, 2679-2689.

Rochefort, C., and Lledo, P.M. (2005). Short-term survival of newborn neurons in the adult olfactory bulb after exposure to a complex odor environment. Eur J Neurosci *22*, 2863-2870.

Rocheteau, P., Gayraud-Morel, B., Siegl-Cachedenier, I., Blasco, M.A., and Tajbakhsh, S. (2012). A subpopulation of adult skeletal muscle stem cells retains all template DNA strands after cell division. Cell *148*, 112-125.

Romero-Aleman, M.M., Monzon-Mayor, M., Yanes, C., and Lang, D. (2004). Radial glial cells, proliferating periventricular cells, and microglia might contribute to successful structural repair in the cerebral cortex of the lizard Gallotia galloti. Exp Neurol *188*, 74-85.

Ross, H.H., Levkoff, L.H., Marshall, G.P., 2nd, Caldeira, M., Steindler, D.A., Reynolds, B.A., and Laywell, E.D. (2008). Bromodeoxyuridine induces senescence in neural stem and progenitor cells. Stem Cells *26*, 3218-3227.

Rugg-Gunn, P.J., Cox, B.J., Lanner, F., Sharma, P., Ignatchenko, V., McDonald, A.C., Garner, J., Gramolini, A.O., Rossant, J., and Kislinger, T. (2012). Cell-surface proteomics identifies lineage-specific markers of embryo-derived stem cells. Dev Cell *22*, 887-901.

Rusakov, D.A., and Kullmann, D.M. (1998). Extrasynaptic glutamate diffusion in the hippocampus: ultrastructural constraints, uptake, and receptor activation. J Neurosci *18*, 3158-3170.

Sahay, A., and Hen, R. (2007). Adult hippocampal neurogenesis in depression. Nat Neurosci *10*, 1110-1115.

Sahin, E., and Depinho, R.A. (2010). Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature *464*, 520-528.

Salter, M.W. (2003). D1 and NMDA receptors hook up: expanding on an emerging theme. Trends Neurosci *26*, 235-237.

Sanai, N., Nguyen, T., Ihrie, R.A., Mirzadeh, Z., Tsai, H.H., Wong, M., Gupta, N., Berger, M.S., Huang, E., Garcia-Verdugo, J.M.*, et al.* (2011). Corridors of migrating neurons in the human brain and their decline during infancy. Nature *478*, 382-386.

Sanai, N., Tramontin, A.D., Quinones-Hinojosa, A., Barbaro, N.M., Gupta, N., Kunwar, S., Lawton, M.T., McDermott, M.W., Parsa, A.T., Manuel-Garcia Verdugo, J.*, et al.* (2004). Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature *427*, 740-744.

Sanchez Alvarado, A. (2000). Regeneration in the metazoans: why does it happen? Bioessays *22*, 578-590.

Scadding, S.R. (1977). Response of adult newt Notophthalmus viridescens to partial or complete gonadectomy. Anat Rec *189*, 641-647.

Scadding, S.R., and Liversage, R.A. (1974). Studies on the response of the adult newt kidney to partial nephrectomy. Am J Anat *140*, 349-367.

Scharff, C., Kirn, J.R., Grossman, M., Macklis, J.D., and Nottebohm, F. (2000). Targeted neuronal death affects neuronal replacement and vocal behavior in adult songbirds. Neuron *25*, 481-492.

Schepers, A.G., Vries, R., van den Born, M., van de Wetering, M., and Clevers, H. (2011). Lgr5 intestinal stem cells have high telomerase activity and randomly segregate their chromosomes. Embo J *30*, 1104-1109.

Seress, L. (1985). Postnatal neurogenesis in the rat hypothalamus. Brain Res *354*, 156-160.

Sexsmith, L.E., and Toronto, O.U. (1968). DNA Values and Karyotypes of Amphibia.

Sheen, V.L., and Macklis, J.D. (1995). Targeted neocortical cell death in adult mice guides migration and differentiation of transplanted embryonic neurons. J Neurosci *15*, 8378-8392.

Shen, Q., Wang, Y., Kokovay, E., Lin, G., Chuang, S.M., Goderie, S.K., Roysam, B., and Temple, S. (2008). Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell *3*, 289-300.

Shi, D.L., and Boucaut, J.C. (1995). The chronological development of the urodele amphibian Pleurodeles waltl (Michah). Int J Dev Biol *39*, 427-441.

Singec, I., Knoth, R., Meyer, R.P., Maciaczyk, J., Volk, B., Nikkhah, G., Frotscher, M., and Snyder, E.Y. (2006). Defining the actual sensitivity and specificity of the neurosphere assay in stem cell biology. Nat Methods *3*, 801-806.

Singleton, A.B., Farrer, M., Johnson, J., Singleton, A., Hague, S., Kachergus, J., Hulihan, M., Peuralinna, T., Dutra, A., Nussbaum, R.*, et al.* (2003). alpha-Synuclein locus triplication causes Parkinson's disease. Science *302*, 841.

Snyder, J.S., Soumier, A., Brewer, M., Pickel, J., and Cameron, H.A. (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature *476*, 458-461.

Song, J., Zhong, C., Bonaguidi, M.A., Sun, G.J., Hsu, D., Gu, Y., Meletis, K., Huang, Z.J., Ge, S., Enikolopov, G.*, et al.* (2012). Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. Nature.

Spalding, K.L., Arner, E., Westermark, P.O., Bernard, S., Buchholz, B.A., Bergmann, O., Blomqvist, L., Hoffstedt, J., Naslund, E., Britton, T.*, et al.* (2008). Dynamics of fat cell turnover in humans. Nature *453*, 783-787.

Spalding, K.L., Bhardwaj, R.D., Buchholz, B.A., Druid, H., and Frisen, J. (2005). Retrospective birth dating of cells in humans. Cell *122*, 133-143.

Speakman, J.R. (2005). Body size, energy metabolism and lifespan. The Journal of experimental biology *208*, 1717-1730.

Spillantini, M.G., Schmidt, M.L., Lee, V.M., Trojanowski, J.Q., Jakes, R., and Goedert, M. (1997). Alpha-synuclein in Lewy bodies. Nature *388*, 839-840.

Stocum, D.L. (1968). The urodele limb regeneration blastema: a self-organizing system. I. Morphogenesis and differentiation of autografted whole and fractional blastemas. Dev Biol *18*, 457-480.

Storch, A., Sabolek, M., Milosevic, J., Schwarz, S.C., and Schwarz, J. (2004). Midbrain-derived neural stem cells: from basic science to therapeutic approaches. Cell Tissue Res *318*, 15-22.

Suomalainen, E. (1950). Pathenogenesis in animals. Adv Genet *3*, 193-253.

Suzuki, M., Yakushiji, N., Nakada, Y., Satoh, A., Ide, H., and Tamura, K. (2006). Limb regeneration in Xenopus laevis froglet. ScientificWorldJournal *6 Suppl 1*, 26-37.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663-676.

Tammenmaa, I., McGrath, J., Sailas Eila, E.S., and Soares-Weiser, K. (2002). Cholinergic medication for neuroleptic-induced tardive dyskinesia. In Cochrane Database Syst Rev (John Wiley & Sons, Ltd).

Tanaka, E.M., and Ferretti, P. (2009). Considering the evolution of regeneration in the central nervous system. Nat Rev Neurosci *10*, 713-723.

Tanaka, E.M., and Reddien, P.W. (2011). The cellular basis for animal regeneration. Dev Cell *21*, 172-185.

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B.B., Siddiqui, A.*, et al.* (2009). mRNA-Seq whole-transcriptome analysis of a single cell. Nat Methods *6*, 377-382.

Tashiro, A., Sandler, V.M., Toni, N., Zhao, C., and Gage, F.H. (2006). NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. Nature *442*, 929-933.

Tattersall, G.J., and Boutilier, R.G. (1997). Balancing hypoxia and hypothermia in cold-submerged frogs. The Journal of experimental biology *200*, 1031-1038.

Taupin, P. (2007). BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. Brain Res Rev *53*, 198-214.

Tavazoie, M., Van der Veken, L., Silva-Vargas, V., Louissaint, M., Colonna, L., Zaidi, B., Garcia-Verdugo, J.M., and Doetsch, F. (2008). A specialized vascular niche for adult neural stem cells. Cell Stem Cell *3*, 279-288.

Taylor, J.H., Woods, P.S., and Hughes, W.L. (1957). The Organization and Duplication of Chromosomes as Revealed by Autoradiographic Studies Using Tritium-Labeled Thymidinee. Proc Natl Acad Sci U S A *43*, 122-128.

Thorndyke, M.C., Patruno, M., Chen, W.C., and Beesley, P.W. (2001). Stem cells and regeneration in invertebrate Deuterostomes. Symp Soc Exp Biol, 107-120.

Toni, N., and Sultan, S. (2011). Synapse formation on adult-born hippocampal neurons. Eur J Neurosci *33*, 1062-1068.

Tozuka, Y., Fukuda, S., Namba, T., Seki, T., and Hisatsune, T. (2005). GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. Neuron *47*, 803-815.

Tramontin, A.D., and Brenowitz, E.A. (1999). A field study of seasonal neuronal incorporation into the song control system of a songbird that lacks adult song learning. J Neurobiol *40*, 316-326.

Treves, A., Tashiro, A., Witter, M.E., and Moser, E.I. (2008). What is the mammalian dentate gyrus good for? Neuroscience *154*, 1155-1172.

Tropepe, V., Craig, C.G., Morshead, C.M., and van der Kooy, D. (1997). Transforming growth factor-alpha null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma. J Neurosci *17*, 7850-7859.

Tsonis, P.A., and Fox, T.P. (2009). Regeneration according to Spallanzani. Dev Dyn *238*, 2357-2363.

Tsonis, P.A., Madhavan, M., Tancous, E.E., and Del Rio-Tsonis, K. (2004). A newt's eye view of lens regeneration. Int J Dev Biol *48*, 975-980.

Valley, M.T., Mullen, T.R., Schultz, L.C., Sagdullaev, B.T., and Firestein, S. (2009). Ablation of mouse adult neurogenesis alters olfactory bulb structure and olfactory fear conditioning. Front Neurosci *3*, 51.

van Hilten, J.J., Ramaker, C.C., Stowe, R., and Ives, N.J. (2007). Bromocriptine/levodopa combined versus levodopa alone for early Parkinson's disease. Cochrane Database Syst Rev, CD003634.

van Praag, H., Kempermann, G., and Gage, F.H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci *2*, 266-270.

van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., and Gage, F.H. (2002). Functional neurogenesis in the adult hippocampus. Nature *415*, 1030-1034.

Vargas, J.P., Lopez, J.C., and Portavella, M. (2009). What are the functions of fish brain pallium? Brain Res Bull *79*, 436-440.

Vellema, M., van der Linden, A., and Gahr, M. (2010). Area-specific migration and recruitment of new neurons in the adult songbird brain. J Comp Neurol *518*, 1442-1459.

Voituron, Y., de Fraipont, M., Issartel, J., Guillaume, O., and Clobert, J. (2011). Extreme lifespan of the human fish (Proteus anguinus): a challenge for ageing mechanisms. Biol Lett *7*, 105-107.

Wagner, D.E., Wang, I.E., and Reddien, P.W. (2011). Clonogenic neoblasts are pluripotent adult stem cells that underlie planarian regeneration. Science *332*, 811-816.

Wagner, G.P., and Misof, B.Y. (1992). Evolutionary modification of regenerative capability in vertebrates: a comparative study on teleost pectoral fin regeneration. J Exp Zool *261*, 62-78.

Wallen, A., and Perlmann, T. (2003). Transcriptional control of dopamine neuron development. Ann N Y Acad Sci *991*, 48-60.

Walton, C., Pariser, E., and Nottebohm, F. (2012). The zebra finch paradox: song is little changed, but number of neurons doubles. J Neurosci *32*, 761-774.

Weiner, W.J., and Reich, S.G. (2008). Agonist or levodopa for Parkinson disease?: ultimately, it doesn't matter; neither is good enough. Neurology *71*, 470-471.

Wigal, S.B. (2009). Efficacy and safety limitations of attention-deficit hyperactivity disorder pharmacotherapy in children and adults. CNS Drugs *23 Suppl 1*, 21-31.

Winner, B., Cooper-Kuhn, C.M., Aigner, R., Winkler, J., and Kuhn, H.G. (2002). Long-term survival and cell death of newly generated neurons in the adult rat olfactory bulb. Eur J Neurosci *16*, 1681-1689.

Witman, N., Murtuza, B., Davis, B., Arner, A., and Morrison, J.I. (2011). Recapitulation of developmental cardiogenesis governs the morphological and functional regeneration of adult newt hearts following injury. Dev Biol *354*, 67-76.

Woodhoo, A., Alonso, M.B., Droggiti, A., Turmaine, M., D'Antonio, M., Parkinson, D.B., Wilton, D.K., Al-Shawi, R., Simons, P., Shen, J., *et al.* (2009). Notch controls embryonic Schwann cell differentiation, postnatal myelination and adult plasticity. Nat Neurosci *12*, 839-847.

Yakushiji, N., Suzuki, M., Satoh, A., Ide, H., and Tamura, K. (2009). Effects of activation of hedgehog signaling on patterning, growth, and differentiation in Xenopus froglet limb regeneration. Dev Dyn *238*, 1887-1896.

Yang, P., Arnold, S.A., Habas, A., Hetman, M., and Hagg, T. (2008). Ciliary neurotrophic factor mediates dopamine D2 receptor-induced CNS neurogenesis in adult mice. J Neurosci *28*, 2231-2241.

Zelenitsky, D.K., Therrien, F., Ridgely, R.C., McGee, A.R., and Witmer, L.M. (2011). Evolution of olfaction in non-avian theropod dinosaurs and birds. Proc Biol Sci *278*, 3625-3634.

Zhang, R.L., Zhang, Z.G., Wang, Y., LeTourneau, Y., Liu, X.S., Zhang, X., Gregg, S.R., Wang, L., and Chopp, M. (2007). Stroke induces ependymal cell transformation into radial glia in the subventricular zone of the adult rodent brain. J Cereb Blood Flow Metab *27*, 1201-1212.

Zhang, R.L., Zhang, Z.G., Zhang, L., and Chopp, M. (2001). Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience *105*, 33-41.

Zhao, C., Deng, W., and Gage, F.H. (2008). Mechanisms and functional implications of adult neurogenesis. Cell *132*, 645-660.

Zhao, C., Teng, E.M., Summers, R.G., Jr., Ming, G.L., and Gage, F.H. (2006). Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J Neurosci *26*, 3-11.

Zhao, M., Momma, S., Delfani, K., Carlen, M., Cassidy, R.M., Johansson, C.B., Brismar, H., Shupliakov, O., Frisen, J., and Janson, A.M. (2003). Evidence for neurogenesis in the adult mammalian substantia nigra. Proc Natl Acad Sci U S A *100*, 7925-7930.

Zhao, S., Rizzolo, L.J., and Barnstable, C.J. (1997). Differentiation and transdifferentiation of the retinal pigment epithelium. Int Rev Cytol *171*, 225-266.

Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J., and Melton, D.A. (2008). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature *455*, 627-632.

Zupanc, G.K., Hinsch, K., and Gage, F.H. (2005). Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. J Comp Neurol *488*, 290-319.